PRECISION-CUT RAT LIVER SLICES IN TOXICITY ASSESSMENT.

IN VITRO TREATMENT WITH IRON (II) SULPHATE AND MENADIONE.

Master thesis in Pharmacology

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June 2007

ACKNOWLEDGEMENTS

I would like to thank my supervisors at GE Healthcare and other personnel who assisted me with laboratory work and analyses, in particular Hubert, Derek, Bente, Mohammed, Jenny-Ann, and Ann.

I must also thank others who volunteered to read and comment on the thesis and helped me bring it to completion: Hans Daniel and Jo. Thank you very much!

Edvard G. Nygaard, June 2007

PREFACE

This thesis is written by Edvard G. Nygaard for the title of pharmaceutical candidate (*cand. pharm.*, or M.Sc. Pharm.) at the School of Pharmacy, University of Oslo. The pharmaceutical study amounts to 5 ½ years, of which 1 year is an individual research project. This research was performed at GE Healthcare AS from October 2000 to November 2001.

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ABSTRACT

In vitro test models may reduce the time and resources required for pre clinical toxicology studies, as well as decrease the number of laboratory animals needed to assess toxicity of drugs. Several models and toxicity assays have been evaluated at GE Healthcare, e.g. using rat liver slices, but the viability of slices has not been optimal and the results inconclusive.

The aims of this study were to test a new incubator and improve the viability of rat liver slices; evaluate various *in vitro* assays for toxicity; and evaluate the *in vitro* toxicity of menadione and iron (II) sulphate on rat liver slices.

Exsanguinous rat livers were cut in slices of 8 mm diameter and 250 µm thickness and incubated in William's medium E with menadione or iron (II) sulphate for various time periods. The slices were analysed for weight, mitochondrial viability (MTT test), enzyme leakage (ALAT, ASAT, GLDH, LDH), and iron and potassium content.

Leakage of certain enzymes (ASAT, GLDH), and reduced potassium content, indicated less viability of liver slices treated with iron (II) sulphate than negative controls. Of the assays studied here, GLDH leakage seemed to be the most predictive of iron (II) sulphate toxicity. The MTT test, LDH leakage, and reduced potassium content indicated less viability of liver slices treated with menadione than negative controls.

More practical training of personnel in using the equipment is required to improve the performance of the rat liver slice model. Isolated hepatocytes may be easier to culture and use for toxicity screening.

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ABBREVIATIONS

Short form	Complete form	Definition, function or use
ALAT, or ALT	alanine aminotransferase	cellular enzyme
ASAT, or AST	aspartate aminotransferase	cellular enzyme
ATP	adenosine triphosphate	energy-storing biomolecule
DMSO	dimethylsulfoxide	organic solvent
FBS	fetal bovine serum	additive to culture medium
GLDH	glutamate dehydrogenase	cellular enzyme
ICP-AES	inductively coupled plasma atomic emission spectrometry	method for quantifying single elements, especially metals
LDH	lactate dehydrogenase	cellular enzyme
MQ-water	milli-Q water	de-ionised RO-water
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide	dye used in assay for cell viability
NADH / NAD^+	nicotinamide adenine dinucleotide (reduced / oxidised)	enzyme cofactor
NBF	neutral-buffered formalin, or normal-buffered formalin	formaldehyde solution adjusted to neutral pH (≈ 7); used to fix tissues for histology
PBS	phosphate-buffered saline	salt water adjusted to physiological pH (7.4) with a phosphate buffer system; used to transport and contain tissue samples during experiments
RO-water	reverse osmosis water	water purified by reverse osmosis
SD	standard deviation	measure of variation within a group of observations (sample size: n), based on the differences between individual data (x) and the sample mean (X) $SD = \sqrt{[\ \Sigma(x-X)^2 / (n-1)\ ]}$ $= \sqrt{[\ (\Sigma x^2 - nX^2) / (n-1)\ ]}$ $= \sqrt{[\ (\Sigma x^2 - \{\Sigma x\}^2/n\ ) / (n-1)\ ]}$
SE, or SEM	standard error of the mean	measure of variation between sample means from different groups, calculated as the SD using the means as individual data, or – if sample sizes are equal and the SDs are the same in all groups – $SE = SD / \sqrt{[n]}$

1. OBJECTIVES

1.1. INTRODUCTION

Validated and relevant *in vitro* models may contribute to reduce the time and resources required for pre-clinical toxicology studies, as well as decrease the number of laboratory animals needed to assess toxicity of drugs.

Several *in vitro* incubation systems and assays for toxicity have been evaluated at the GE Healthcare site in Oslo. For example rabbit kidney slices and rat liver slices have been cultured for periods of up to 24 hours, but the viability of slices has not been optimal and the results inconclusive. One of the goals of this project was therefore to improve the quality and viability of slices in the liver slice model.

A new incubator had been purchased recently, allowing tissue slices to be incubated in rolling glass vials with teflon or titanium inserts. The intention was to evaluate different concentrations and types of test substances, and different exposure times, as well as various *in vitro* assays for toxicity, e.g. enzyme leakage, the MTT test, and histology.

A known hepatotoxicant, menadione, was to be used as the test substance in early experiments, and as a positive control for one or more test substances in later experiments. A substance of interest in the later experiments was iron, which is the principal constituent of certain magnetic resonance imaging (MRI) contrast agents currently in development. These agents contain microparticles of iron oxide, but for testing of toxicity a soluble form of iron was chosen for this project; iron (II) sulphate (ferrous sulphate, $FeSO_4$).

Rat livers, liver slices and isolated hepatocytes, and various iron compounds including iron (II) sulphate, have been involved in previous research on iron toxicity (e.g. Bacon *et al.* 1986, Mak and Weglicki 1985, Pushpendran *et al.* 1998). However, the treatment of rat liver slices with iron (II) sulphate seems to be a novel approach.

1.2. PRIMARY OBJECTIVES

- test the new incubator and improve the quality and viability of slices in the liver slice model at GE Healthcare
- evaluate various in vitro assays for toxicity, e.g. enzyme leakage and the MTT test, to identify the most sensitive endpoints

1.3. SECONDARY OBJECTIVES

- observe the effects of different exposure times and concentrations of test substances
- evaluate the in vitro toxicity of menadione and iron (II) sulphate on rat liver slices

2. BACKGROUND

2.1. HEPATOTOXICITY

2.1.1. General aspects

The liver is the largest internal organ in mammals and is richly supplied with blood from the hepatic artery and the portal vein, filtering approximately one fifth of cardiac output each minute. All blood from the intestines is drained through the portal vein, and since the majority of xenobiotics enter the body via the gastrointestinal tract and are absorbed into the blood by the intestines, they must first pass the liver to reach the systemic circulation. Considering its size and position, in addition to its very high concentrations of a variety of enzymes, the liver is the major metabolic and detoxifying organ of the body. However, its anatomy and blood supply also makes it vulnerable to toxic influences, and injury to the liver may have profound systemic repercussions.

Most xenobiotics are oxidised or otherwise converted to harmless (or less harmful) substances by the liver. It facilitates their excretion via the kidneys through increasing their water-solubility, or takes them out of circulation by complexing them with bile salts and excreting them into the bile duct and thence into the intestine. Some compounds may be activated rather than inactivated by hepatic enzymes. Others cause transformations of the enzymes themselves, destroying their function and reducing the liver's metabolic capacity until new enzymes have been synthesised. The liver may also fail to metabolise the compounds rapidly enough, allowing them to persist and cause toxicity to the liver, as well as to other organ systems.

Cytotoxic mechanisms include cytoskeleton alterations, mitochondrial dysfunction and energy deprivation, loss of thiols and antioxidant status, and perturbation of intracellular calcium homeostasis with subsequent activation of degradative enzymes. Damage to the plasma membrane or a block in ATP synthesis impairs energy-dependent cellular ion pumping mechanisms, allowing sodium and calcium to enter and potassium to escape, resulting in loss of plasma membrane volume control and acute cell swelling (Vickers, 1997). The liver can generally recover from mild acute injury by hepatocellular regeneration with the production of new cells, which restore liver function and normal tissue architecture, but

chronic or more severe acute injury may lead to fibrogenesis, scar formation, and distortion of the normal tissue architecture (Mehendale, 1991).

Endpoints of chronic toxicity observed in liver include cell necrosis, apoptosis, steatosis (fatty liver), cholestasis (inhibition of bile flow) and jaundice, hepatitis, cirrhosis, and neoplasia (Hodgson, 2001; Plaa and Charbonneau, 1994). Because cellular necrosis is accompanied by leakage of cellular contents, their detection in plasma or serum is commonly used as a non-invasive surrogate endpoint. Some enzymes measured to monitor liver status are alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) (Meyer, 2001).

2.1.2. Menadione

Menadione is a member of the vitamin K family (Figure 2.1-a), which is involved in the bio-activation, or post-translational modification, of blood coagulation factors II (prothrombin), VII, IX, X, and certain other proteins. *In vivo*, both menadione (vitamin K_3) and phyllo-quinone (vitamin K_1) are converted to the bioactive metabolite menaquinone-4 (vitamin K_2). This form of the vitamin is a coenzyme for the oxidation of glutamic acid (Glu) residues to γ-carboxyglutamic acid (Gla) in the proteins mentioned earlier (Merck Index, 1996).

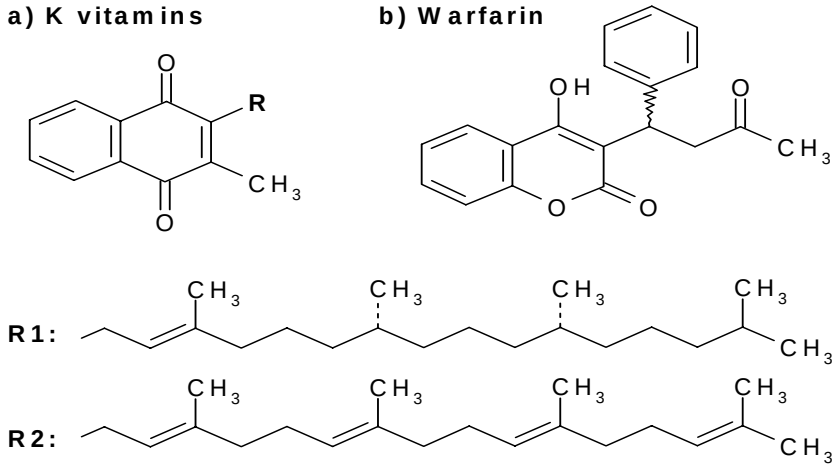


Figure 2.1. Chemical structures of a) some K vitamins including menadione, and b) the vitamin K antagonist, warfarin

$R = R_1$	*vitamin K_1*	*phylloquinone*	*2-methyl-3-phytyl-1,4-napthoquinone;*
$R = R_2$	*vitamin K_2*	*menaquinone-4*	*2-methyl-3-tetraprenyl-1,4-napthoquinone;*
$R = H$	*vitamin K_3*	*menadione*	*2-methyl-1,4-napthoquinone.*

Vitamin K is used therapeutically as a pro-thrombogenic drug and as an antidote for poisoning with coumarin derivatives. Some examples of these are the sweet clover

constituent bishydroxycoumarol, which causes sporadic casualties in livestock, and warfarin (Figure 2.1-b), the active principle of rat poison and some anticoagulant drugs. On the other hand, excessive vitamin K is itself toxic due to its oxidative properties, and because of its lipid solubility it may accumulate in adipose tissue. The toxicity of menadione and other K vitamins involves oxidative stress caused by redox cycling and/or arylation of protein thiols (Stubberfield and Cohen, 1989).

Although normally metabolised to menaquinone *in vivo*, menadione does not need any metabolic activation to exert its toxicity and is commonly used as a positive control substance for *in vitro* toxicology studies (Leeman *et al.,* 1995; Price *et al.,* 1996). However, a certain level of oxygen (O_2) is necessary to reveal the toxicity of menadione. In isolated hepatocytes treated with 100-400 μM menadione, the threshold level lies below the atmospheric oxygen average of 21% (Utley and Mehendale, 1989). 200 μM menadione at 21% O_2 is toxic to hepatocytes but not to liver slices (Wright and Paine, 1992). Toxic effects are observed in liver slices treated with 50-400 μM menadione at 40% O_2 (Leeman *et al.,* 1995), or with 100-300 μM menadione at 95% O_2 (Chan *et al.,* 1992).

The liver slices in the present study were supplied with 95% O_2 / 5% CO_2 during incubation, and the medium had also been gassed with 95% O_2 / 5% CO_2 before incubation.

2.1.3. Iron

Iron (Fe) is an essential element for most living organisms, and in many higher forms of life it comprises the central part of the oxygen-carrying haemoglobin molecules of the red blood cells. It is also part of a number of electron-carriers in the respiratory chain, plus many other enzymes. Iron is transported throughout the body by transferrin, a specialised iron-carrying protein, and is stored intracellularly bound to ferritin, mainly in the liver.

Varying amounts of iron are lost in menstrual bleeding, haemorrhage, and red cell turnover, and must be replaced through the diet. Cereals, coarse bread, liver and blood products are rich sources of iron. If the diet does not replace all the iron lost or the iron is not properly exploited, which may be the case for fertile women, elderly people and in certain diseases, a supplement of iron should be taken. However, iron is also a highly reactive element that promotes free radical formation, and in high concentrations it is carcinogenic (Griffiths *et al.,* 1999). It can therefore be dangerous to take an iron supplement constantly without

monitoring the iron level, or to take large doses of iron at any one time. Regulation of *in vivo* iron levels concerns only uptake, and the most common disorders involving misregulation result in hyperchromatosis, i.e. iron overload (Griffiths *et al.,* 1999).

The liver is a target organ for iron accumulation and toxicity in various iron-overload disorders, and *in vitro* oxidative damage by ferrous iron has been demonstrated in liver homogenates (Pushpendran *et al.,* 1998), isolated hepatic lysosomes (Mak and Weglicki, 1985), and isolated hepatic mitochondria (Bacon *et al.,* 1986; Pushpendran *et al.,* 1998). The isolated perfused rat liver, even when it is already in a state of iron overload, effectively takes up and clears non-transferrin-bound iron from plasma (Wright *et al.,* 1986; Wright and Lake, 1990).

Ferrous iron (Fe^{2+}) is directly toxic, whereas the toxicity of ferric iron (Fe^{3+}) depends on conversion to ferrous iron by a reducing agent such as ascorbic acid (vitamin C), or cycling between ferric and ferrous iron (Mak and Weglicki, 1985). Because of its influence on the toxic potential of iron, the common antioxidant vitamin C may in large doses (several grams) prove harmful to persons affected by hyperchromatosis of various aetiologies. Although the incidence of chronic iron overload is well below 1%, caution should be exhibited regarding high-dose vitamin C.

2.2. *IN VITRO* LIVER CULTURE SYSTEMS

To minimise the number of animals used in toxicological studies, and to improve the utilisation of time and other resources in such studies, an array of *in vitro* models have been developed in different laboratories. These methods are used, among other things, for metabolism studies (phase I and phase II metabolism), toxicity screening (which compounds are toxic and which are not), comparative medicine (comparing species), and for determining the appropriate species for *in vivo* studies. A few *in vitro* systems for culturing liver tissue will be described here; the isolated perfused liver, isolated hepatocytes, and liver slices. Some assays for toxicity are described in section 2.4 (page 24 ff) and section 3.3 (page 40 ff).

As mentioned in section 2.1.1, the hepatotoxic potential of a compound is often related to its biotransformation. An *in vitro* toxicological system should therefore reproduce the *in vivo* biotransformations of compounds in order to be predictive of hepatotoxicity. However, the

in vivo metabolism and effects of many substances vary according to inter-individual differences in enzyme levels, health condition, alcohol or drug abuse, the legitimate use of drugs, and other factors not easily reproduced *in vitro*. Results from *in vitro* experiments should be interpreted with these potentially confounding factors in mind. On the other hand, relative homogeneous *in vitro* study materials available from animals of the same age, sex, and breed, may contribute valuable stability to routine investigations of large amounts of test compounds. Other advantages and disadvantages of *in vitro* systems are discussed below.

2.2.1. The isolated perfused liver

The liver is excised together with its afferent and efferent blood vessels, then submerged in a suitable oxygenated, buffered and temperature-controlled nutrient medium, with the blood vessels connected to tubes. By use of pumps and these tubes, the organ is provided with a perfusate similar to the surrounding medium, and this is collected for analyses after passage through the liver. It may also be recycled through the liver continuously or a specific number of times. Various substances can easily be added to the afferent perfusate and changes in composition, reflecting metabolic activity in the organ, can be monitored.

Important advantages of the perfused organ model are, preservation of cellular and whole-organ functionality, a very good *in vivo – in vitro* correlation, and ease of administration and collection of test substances. Unfortunately the regional, cellular, and sub-cellular locations of particular biochemical reactions are difficult to establish, and the viability of organs can only be sustained for relatively short periods. Other disadvantages include limited numbers of experimental units (only one liver per animal), and large laboratory space and perfusate volumes needed per unit (often several litres).

2.2.2. Isolated hepatocytes

The liver consists of parenchymal cells, or hepatocytes, and non-parenchymal cells including Kupffer cells, biliary epithelial cells, blood vessel endothelial cells, and several other cell types. Much of the work on isolated liver cells has been focused on hepatocytes because they are the main metabolic unit of the liver, and they amount to 60% of the organ's cells by number; 80% by volume (Plaa and Charbonneau, 1994). To isolate hepatocytes, the liver is perfused with an oxygenated collagenase-containing buffer, which must be sterile and bubble-free. Collagenase digests the extracellular matrix in the liver without disrupting

cellular membranes or Glisson's capsule, the outer membrane of the organ. This procedure can either be performed on a previously excised liver stored on wet ice in a sterile environment for up to 36 hours, or *in situ* during non-survival surgery (Mudra and Parkinson, 2001). The latter method yields a higher percentage of viable hepatocytes, but requires a highly skilled technician to avoid maltreatment of the test subject.

After digestion with collagenase the hepatocytes are suspended in sterile, oxygenated, buffered nutrient medium, then filtered. The suspension is centrifuged, the supernatant removed, and the cells resuspended in new medium several times to thoroughly rinse the cells and to remove dead cells which will float during centrifugation. The number of cells per ml is counted after staining a sample of the suspension with trypan blue, and the rest of the suspension is diluted with medium to the desired concentration (e.g. $1x10^6$ cells/ml). Aliquots of hepatocytes can be cryo-preserved or used directly in experiments, in which case they are seeded into 24- or 96-well microplates, or larger vials, with medium containing test substances of interest. Incubation is done at 37°C for the desired time, and the necessary analyses are performed (Mudra and Parkinson, 2001).

Among the advantages of isolated hepatocytes are the high numbers of cells obtained, their ability to survive cryo-preservation, and their maintenance for longer periods than whole organs; at least 6 hours. However, when the extracellular matrix is disrupted and the hepatocytes are separated, they rapidly de-differentiate and do not retain the regional specialisation within the liver lobes (periportal, centrilobular, canalicular).

An extension of the isolated hepatocyte method is the use of hepatocyte cultures permitting longer test periods by attaching cells to an artificial extracellular matrix placed in a periodically refreshed medium to keep them viable (Berry *et al.,* 1991; Grisham, 1979). Factors such as the matrix used for cell attachment, culture medium, addition of hormones, oxygen tension, cell density, and presence of other cell types determine enzyme and gene expression and affect cell viability in mono-layer cultures of hepatocytes (McQueen, 1993).

2.2.3. Liver slices

Earlier, tissue slices were prepared by hand using razorblades, but nowadays they are prepared with mechanical precision instruments (for details on procedures, see the next section). Slices are generally prepared from untreated animals and exposed to the study drug or drugs *in vitro*, although a few experiments are performed with pre-treated organs.

Several factors make the use of tissue slices attractive. The major advantage of tissue slices over single-cell systems is that the slices contain all cell types of an organ in their normal *in vivo* architectural relationships with the preservation of intercellular communication and interactions, enabling both regional and cell-specific effects to be investigated (Bach *et al.,* 1996; McGuinness *et al.,* 1993; Ruegg, 1994). Compared to whole-organ systems slices require less laboratory space per experimental unit, and are not as limited in number. As many as 75-100 slices may be obtained from an adult rat liver, or 10000-20000 slices from an adult human liver (Bach *et al.,* 1996). Tissue slices also facilitate comparison between different organs and species, since the methodology for preparing and treating slices is similar regardless of the organ or species from which the slices are derived (Bach *et al.,* 1996; Gandolfi *et al.,* 1995). Last, but not least, incubations of tissue slices can last for 5 days under sterile and otherwise strictly controlled conditions (Bach *et al.,* 1996; Fisher *et al.,* 1995a; Parrish *et al.,* 1995; Smith *et al.,* 1985).

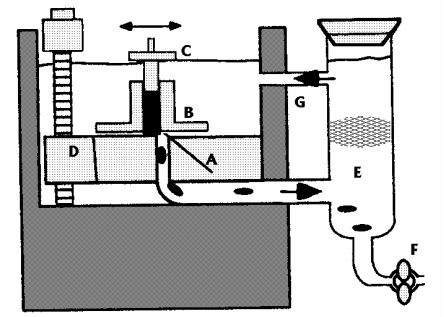
Compared to *in vivo* methods, advantages of tissue slices involve decreased costs, time, and numbers of animals necessary to complete a study.

2.3. PRECISION-CUT TISSUE SLICES

2.3.1. Preparation of slices – the Krumdieck tissue slicer

When tissue slice techniques were first introduced, the cutting of slices by hand led to very heterogeneous sets of slices. In addition, the manual process rendered slices too thick to allow sufficient oxygen and nutrient supply for the inner cell layers, resulting in necrosis (Smith *et al.,* 1985). Later, mechanical tissue slicers have been developed, capable of producing thin tissue slices of consistent thickness from a range of organs and species. One of these, the Krumdieck tissue slicer, is described here.

In 1980, Krumdieck *et al.* had developed a mechanical tissue slicer that allowed a relatively inexperienced operator to rapidly produce slices of nearly identical thickness, of very similar shape, with minimal tissue damage, and in a controlled environment. The Krumdieck tissue slicer (Figure 2.2) contains a motorised, horizontally oscillating razorblade submerged in a reservoir filled with a viscous, oxygenated buffer. The buffer circulates through channels in the reservoir, driven by the centrifugal force of the blade's power shaft. Cylindrical tissue cores resting on a horizontal base are held in place by a cutting arm with weights, and moved back and forth across the oscillating blade by the movement of the arm, which is also motorised. A tissue slice is produced on each passage of the core against the razorblade. Slice thickness is controlled with a micrometer screw regulating the vertical distance between the blade and the base upon which the cores rest, and the achieved thickness corresponds well to the setting of the instrument (Krumdieck *et al.*, 1980). Optimal dimensions of slices vary with regard to organs and species, but for rat liver slices the optimal thickness is 200-250 μm (Smith *et al.*, 1985) and diameter 8 mm (Fisher *et al.*, 1995b).



Slices are produced by a mechanically driven, straight razorblade (A), which oscillates in a plane perpendicular to the diagram. The tissue core rests in the cutting arm (B) and is swept across the blade by a piston pivot mount. Slice thickness is a function of tissue type, the weight over the tissue core (C), and the setting of the micrometer screw (D). Slices are trapped on a strainer placed in the slice collection vessel (E) and are retrieved through a valve during breaks in motor operation (F). Slicing buffer is returned to the reservoir (G) after passing through the collection vessel. Diagram modified with permission from Parrish et al. (1995).

Figure 2.2. Schematic view of the Krumdieck tissue slicer (Alabama R&D)

One problem with early mechanical slicers was that the process of removing ready-cut slices was slow and very dependent on the experience of the operator. This is not a problem with the Krumdieck tissue slicer because slices are carried away by the circulating buffer and trapped on a strainer from which they can be removed at intervals and stored on ice. Thus, the operator's task is simplified and the instrument's function is speeded up, and one can easily obtain more than 10 slices per minute. Another major advantage of the Krumdieck tissue slicer is that the tissue is constantly bathed in oxygenated, buffered medium, which may also be cooled during the operation by adding a recirculating refrigeration unit, improving the viability of slices. A study performed on the variation of liver slices produced

by the Krumdieck tissue slicer demonstrated a consistency among slices with regard to thickness and surface area, allowing them to be used as individual experimental units (Smith *et al.,* 1985).

2.3.2. Incubation of slices

2.3.2.1. Time span

The incubation of slices can roughly be divided into three categories (Bach *et al.,* 1996):

(a) Short-term incubation; slices are incubated with various compounds for 8 hours or less in simple tissue culture media. These conditions are often suitable for investigating the metabolism of compounds.

(b) Intermediate-term incubation; slices are incubated for up to 24 hours in nutrient-enriched tissue culture media for studies on toxicity and metabolism. Slices can also be exposed to chemicals for a short time and then maintained for up to 24 hours to assess the effects of acute exposure.

(c) Long-term incubation; slices are kept viable for more than 24 hours in specially enhanced tissue culture media and under strict control of physico-chemical and micro-biological environment. This makes it possible to investigate the effects of longer exposure to chemicals and to study induction of enzymes.

In the present study, liver slices are incubated on short and intermediate term.

2.3.2.2. Incubation systems

A variety of incubation systems have been developed for slices, generally based on two principles; the continuously submerged incubation and the dynamic organ culture incubation. Both principles have their unique attributes, but their common feature is the delivery of nutrients and oxygenated culture medium to both surfaces of the slices.

During continuously submerged incubation, slices are in the medium all the time and air or other gas supply is bubbled through or passed over the medium. Some examples are the submersion bubble system (Ruegg *et al.,* 1987), the stirred well system (Dogterom, 1993), and the shaken flask system (de Kanter and Koster, 1995). Using continuously submerged

incubation, viability of liver slices has been maintained for 1 to 3 days, and some of these systems are suitable for long-term incubation (Bach *et al.,* 1996).

In dynamic organ culture incubation, slices are placed on small-meshed nets and intermittently exposed to medium and air (gas), improving the oxygen transfer into slices. Examples are the dynamic roller system (Smith *et al.,* 1985) and the rocker platform system (Leeman *et al.,* 1995). Incubations preserving viability for up to 5 days have been performed using this principle, and it is believed to be the optimal one for long-term incubation of liver slices (Bach *et al.,* 1996; Fisher *et al.,* 1995a; Parrish *et al.,* 1995).

The present study uses dynamic organ culture incubation with rolling vials (see Figure 3.4, page 34).

2.3.2.3. Culture media

A number of nutrient-enriched culture media have been tested to find the optimal media for incubating tissue slices from different organs, and various synthetic or semi-synthetic media have been developed especially for liver slices. One research group found that all the culture media they tested – DMEM, DMEM/F12, MEM, H-Y, McCoy's, RPMI, Waymouth, and Williams' E – maintained cellular concentrations of potassium (K) above 80 µmol per gram wet weight for 72 hours and were suitable for long-term incubation of rat liver slices (Fisher *et al.,* 1995b). All the tested media were without phenol red and were supplemented with sodium bicarbonate, 10% fetal calf serum, 85 µg/ml gentamicin sulphate, and 1% FungiBact.

The present study uses Williams' medium E with phenol red, sodium bicarbonate, 5% fetal bovine serum and 1% Antibiotic-Antimycotic, in addition to L-glutamine, glucose, and insulin (for more details on medium composition see section 3.2, page 32 ff).

2.4. MARKERS OF TOXICITY

2.4.1. Lipid peroxidation and antioxidant status

The integrity of cellular membranes (plasma membrane, mitochondrial membranes, nuclear envelope, etc.) depends heavily on the chemical stability of their lipid constituents, mainly cholesterol, triglycerides, and phospholipids. Reactive oxygen species and other free radicals involved in toxicity may create an oxidative stress disturbing cellular antioxidant status, particularly by oxidation or arylation of thiols such as glutathione, so that the lipids and other bio-molecules protected by the thiols become vulnerable to oxidation.

The extent of lipid peroxidation is assessed by the production of malondialdehyde (MDA) using the thiobarbituric acid (TBA) test. Samples are treated with a solution of TBA, and MDA is then determined spectrophotometrically at 535 nm (Burge and Aust, 1978). Anti-oxidant status can be established by measuring both reduced (GSH) and oxidised (GSSG) forms of glutathione using Hissin and Hilf's method (1976) or a modification thereof (Obatomi *et al.,* 1998a). The assay involves fluorescence spectrometry with excitation at 350 nm and emission at 420 nm.

2.4.2. Alterations in protein synthesis

Toxic substances affect protein functionality in a number of ways, ranging from the early steps of gene transcription (DNA template; RNA synthesis) via gene translation (RNA template; protein synthesis) to protein performance and in some cases the excretion of proteins from a cell. Through chemical bonding and chemical reactions the toxins may inhibit the function or cause the destruction of DNA, RNA, the proteins themselves, or the enzymatic machinery associated with them.

The method of Downs and Wilfinger (1983) or later modifications can be used to measure cellular content of intact DNA. Samples are dissolved in a buffered mixture of surfactants and chelators, and DNA content is determined fluorometrically with excitation at 350 nm and emission at 460 nm (Miller *et al.,* 1993).

Protein synthesis and excretion can be measured after incubation with a radioactively labelled amino acid, e.g. 0.3 μCi/ml of L-[^{3}H]leucine. The incorporation of radioactivity into acid-precipitable protein is assessed by liquid scintillation counting and expressed as

dpm/mg protein (dpm; number of radioactive degradations per minute) (Fisher *et al.,* 1995b; Sipes *et al.,* 1987; Smith *et al.,* 1986). For the protein synthesis assay the whole sample is dissolved before precipitation of protein, whereas only culture-medium proteins are precipitated for the excretion assay. Total protein is routinely determined according to Lowry *et al.* (1951).

Excretion or leakage of certain enzymes, which are proteins, was measured in the present study using photometric methods (see the next section).

2.4.3. Enzyme leakage

Cytotoxic mechanisms include cytoskeleton alterations and lipid peroxidation, compromising the integrity of the plasma membrane. This may induce cellular swelling or necrosis and is accompanied by leakage of cellular contents into the extracellular space. In the earlier stages of tissue damage, cytoplasmic enzymes may leak from cells where plasma membrane permeability has altered. As the severity of tissue damage progresses, enzymes normally present in subcellular organelles will be released (Evans GO, 1996). If the leakage adds significantly to the extracellular levels of particular substances, which is often the case with cellular enzymes, it can be detected in blood plasma *in vivo*, or in culture medium *in vitro*, and is a marker of toxicity.

Several tissue- or organ-specific enzymes have been identified making distinctions feasible between various illnesses or injuries with only blood samples instead of biopsies. *In vitro* toxicological studies on kidney or liver almost invariably include measurements of lactate dehydrogenase (LDH), although this enzyme is not particularly organ-specific. Other, more specific candidates are alkaline phosphatase (ALP) for kidney studies and alanine amino-transferase (ALAT) or aspartate aminotransferase (ASAT) for liver studies (Obatomi *et al.,* 1998b). However, the organ specificity of an enzyme may differ between species, or even individuals, which must be considered in the choice of enzymes and analytical methods in comparative medicine.

An array of commercial reagent kits exist for measuring concentrations of various enzymes in different matrices, mainly serum or plasma. The methods of quantification are often based on the rate of oxidation of NADH to NAD^+ (two forms of the coenzyme, nicotinamide adenine dinucleotide) being linearly correlated with enzyme concentration, and the dis-

appearance of NADH detectable by UV-absorption photometry. The contents and procedures of the reagent kits are generally recommended by the International Federation of Clinical Chemistry (IFCC), the French Society of Clinical Biology (Société Française de Biologie Clinique, SFBC), the German Society of Clinical Chemistry (Deutsche Gesellschaft für Klinische Chemie, DKGC), or other major national associations.

In the present study, the activities of four enzymes are measured in the incubation media after incubation of rat liver slices for 3, 6, or 24 hours:

- alanine aminotransferase (ALAT), liver-specific in rats, mainly cytosolic;
- aspartate aminotransferase (ASAT), less liver-specific, cytosolic/mitochondrial;
- glutamate dehydrogenase (GLDH), liver-specific in rats, mainly mitochondrial; and
- lactate dehydrogenase (LDH), low organ-specificity, mainly cytosolic.

ALAT (also known as glutamic pyruvic transaminase, GPT) (Enzyme Commission no. EC 2.6.1.2) is mainly a hepatic enzyme in many species, e.g. rat and dog, and plasma levels of the enzyme can be measured as a marker of liver status or hepatic injury. It is generally considered a cytosolic enzyme, but is also present in the mitochondria of some species, including the rat (Evans GO, 1996).

ASAT (also known as glutamic oxaloacetate transaminase, GOT) (Enzyme Commission no. EC 2.6.1.1) is less liver-specific than ALAT, but is often measured together with ALAT to identify the site and extent of injury. It exists in both mitochondria and cytosol; the proportion of mitochondrial versus cytosolic forms being greater with ASAT than with ALAT (Evans GO, 1996).

GLDH (Enzyme Commission no. EC 1.4.1.3) is an essentially mitochondrial enzyme present in liver, kidney and muscle tissues, with the highest concentrations in liver (Evans GO, 1996).

LDH (Enzyme Commission no. EC 1.1.1.27) is a cytosolic enzyme widely distributed in different tissues, the distribution patterns varying from species to species. In rats the enzyme is not specific to the liver, but may indicate general tissue damage (Evans GO, 1996).

2.4.4. Reduced mitochondrial viability – the MTT test

As mentioned above, mitochondrial membranes are vulnerable to oxidative stress and lipid peroxidation. The components of the electron transport chain (ETC) of cellular respiration are located in these membranes and are also vulnerable to oxidation, and injury to either the membranes or the ETC may result in mitochondrial dysfunction. Examples of ETC components are ubiquinone (coenzyme Q_{10}), ferredoxin, cytochromes a, b, c, and various dehydrogenase enzymes.

The water-soluble yellow dye, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide, or $C_{18}H_{16}N_5SBr$, so-called thiazolyl blue), is absorbed by cells and reduced to the water-insoluble blue dye, formazan ($C_{18}H_{17}N_5S$), by dehydrogenase activity in functional mitochondria (Figure 2.3). For a given cell type, the quantity of formazan produced is proportional to the number of living cells and the concentration of MTT. The formazan is extracted with an organic solvent such as DMSO or isopropanol, and the optical density (OD) of the resulting solution is measured photometrically at a wavelength of 505, 550, or 570 nm. If required, reference values may be obtained from untreated cells. All values should be normalised against protein content or tissue weight (Berridge and Tan, 1993; Borenfreund *et al.,* 1988; Leeman *et al.,* 1995; Mosmann, 1983; Obatomi *et al.,* 1998c).

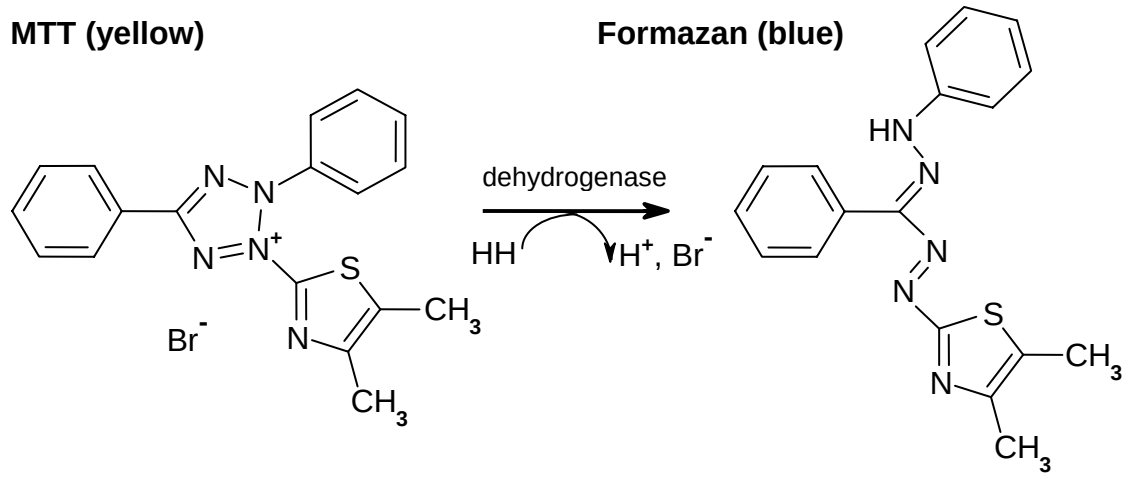


Figure 2.3. Reaction scheme for the reduction of MTT to formazan

This reaction is catalysed by dehydrogenase in functional mitochondria. Two ground-state hydrogen atoms represent the enzyme's catalytic site, and the by-products of the reaction are bromide and hydrogen ions.

2.4.5. Potassium leakage and reduced ATP levels

Entry and exit of potassium (K) to and from cells is central to many physiological processes, e.g. stomach acid production, hormone secretion, neurological action potentials, and muscular contraction. Cellular K levels are normally maintained by the energy-dependent Na/K-ATPase, and lowered by voltage- or substrate-controlled ion channels or other ion exchange mechanisms. Damage to the plasma membrane or a block in ATP synthesis impairs these mechanisms, allows sodium and calcium to enter and potassium to escape, and causes acute cell swelling (Vickers, 1997). Production of ATP takes place in the mitochondria and may be inhibited by processes mentioned in the previous section, or by an insufficient supply of nutrients. The ATP level in a cell thus reflects both cellular integrity and the metabolic state of the cell.

Potassium leakage from cells or tissues is not easily measured. However, the retained intracellular content of the element can be determined by flame photometry, or atomic absorption- or emission-spectrophotometry (AAS or AES). Samples are washed in 0.9% (w/v) NaCl, homogenised e.g. by sonication, and dissolved in strong acid, before measuring the concentration of K in the resulting solution (Dogterom, 1993; Price *et al.,* 1998; Smith *et al.,* 1986).

ATP can be quantified by the ATP-dependent, bioluminescent luciferin-luciferase reaction. The tissue is homogenised and centrifuged, and the supernatant treated with a luciferin-luciferase solution. Bioluminescence is measured in a luminescence photometer, and the result is given as amount of ATP per milligram of protein (DeLuca and McElroy, 1978; Kimmick *et al.,* 1975; Lundin *et al.,* 1986; Lundin, 2000). Alternatively, ATP and the other adenosine nucleotides (ADP, AMP) can be quantified by high-performance liquid chromatography, HPLC (Dogterom, 1993; Jones, 1981; Olinga *et al.,* 1997).

3. MATERIALS AND METHODS

The origin of a material is generally specified in parentheses after the first appearance of the material in a new context. The specifications always include name of brand or supplier, which may or may not be abbreviated, and in most cases an article/catalogue number or other unique type designation is also given. (See appendix A–1 for a more detailed list of manufacturers and suppliers.)

3.1. PREPARATION OF RAT LIVER SLICES

3.1.1. Animals

Livers from 6-8 weeks old, male Sprague-Dawley rats (type BKL from B&K) were used. (See appendix A–2 for a summary of animal characteristics.) The animals were acclimatised for at least 5 days at a temperature of 21±2°C, relative air humidity 55±10%, ventilation changing the air 20 times per hour, and periods of 12h light / 12h dark in phase with natural daylight. Standard food (811002 / RM1(E) SQC from SDS) and quality-controlled tap water were supplied *ad lib* until 8 or 9 p.m. on the last night, at which time food was withdrawn. Water was not withdrawn, but rats usually do not drink much when not eating.
The experiments started between 8 and 9 a.m.

In each experiment one animal was used, except from one occasion when two animals of the same litter were used in order to have more liver material (experiment denoted EGN-05-01 in appendix A–2). To simplify matters, however, methods will principally be described and results presented so that each experiment can be interpreted as involving one animal.

Most of the rats were decapitated by means of a guillotine (AH-55-0012 from Harvard), to which the animals had been trained during acclimatisation. In the first few experiments involving menadione alone (section 3.2.2) the animals were not decapitated, but had their neck arteries cut during isoflurane anaesthesia (Isoflurane from Baxter).

3.1.2. Preparation of liver cores

In each experiment the liver was excised, weighed, and transported to the laboratory in ice-cold PBS (phosphate-buffered saline)* which had been gassed with 95% oxygen / 5% carbon dioxide (5 mole-% carbon dioxide in oxygen, from AGA) for 10 minutes immediately before use. Liver weights were between 7.4 and 9.8 grams (see appendix A–2). The wet liver was placed on 3-4 layers of wet filter paper (331511 / 520B from Schleicher) on top of a plastic support. Using a coring tool composed of a sharpened, 8 mm diameter, stainless steel tube (MP-0144 from Alabama) and an electric drill (6470H2 from Skil) mounted vertically in a levered rack, 8 mm tissue cores were prepared from all liver lobes that were sufficiently wide (Figure 3.1-a). The steel cylinder, filter papers, and liver were moistened with PBS at intervals during the process.

Preparation of cores started 5-10 minutes after excising the liver and was finished within an additional 15-25 minutes. The cores were collected in ice-cold PBS which had been gassed with 95% O_2 / 5% CO_2. Suitable cores were approximately cylindrical; that is, circular and not too wedge-shaped, and having nearly parallel cutting edges (Figure 3.1-b). To spread lobe variations, cores from different lobes were mixed to some degree before the liver slices were prepared.

* PBS: Dissolve 5 PBS tablets (P-4417 from Sigma) in 1 litre of sterile water (82479-E from Braun) to obtain ≈9.5 mM phosphate buffer (8.10 mM Na_2HPO_4 and 1.47 mM KH_2PO_4), 2.68 mM KCl and 137 mM NaCl; pH 7.4. Keep at 4°C for up to 1 week.

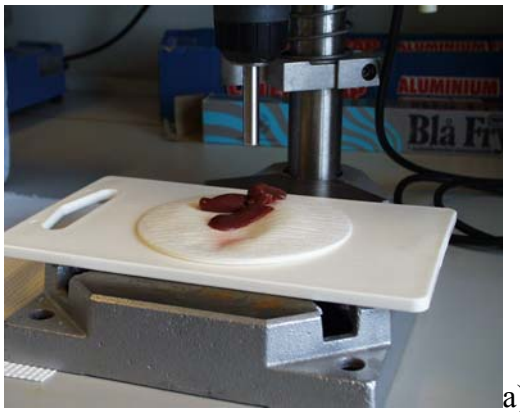
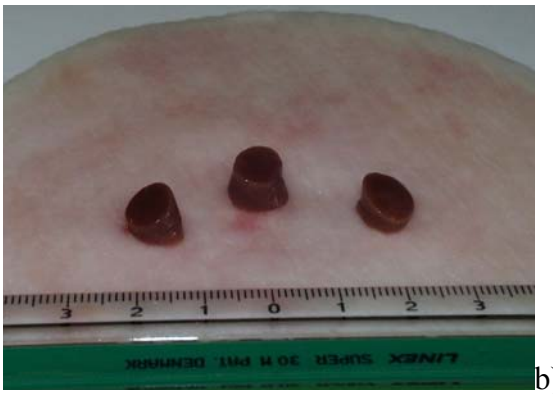

a)

b)

Figure 3.1. a) Rat liver and coring tool. b) Examples of liver cores (scale: cm, mm)

3.1.3. Preparation of liver slices

Slices were prepared from the liver cores using a Krumdieck tissue slicer (MD-1100-A2 from Alabama) (Figure 3.2-a; see also Figure 2.2, page 21) set to a nominal slice thickness of 240 µm and a cycle speed of 60 per minute (i.e. the frequency of movements across the razorblade). The microtome and reservoir of the slicer had been cleaned, assembled and refrigerated to 4°C before use, disinfected by spraying with 70% ethanol (from Arcus), run through with two portions of ice-cold, sterile water (82479-E from Braun), and was water-cooled during the slicing process. The reservoir was filled with ice-cold slicing buffer consisting of PBS with 0.08% to 0.1% (w/v) agarose† (Obatomi *et al.,* 1998a, c), which was gassed with 95% O_2 / 5% CO_2 for 10 minutes immediately before use.

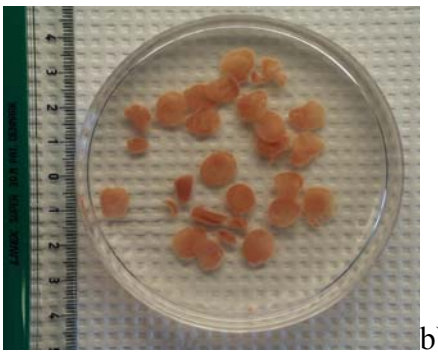
a)

b)

Figure 3.2. a) Front view of tissue slicer. b) Examples of liver slices (scale: cm, mm)

† Slicing buffer: Add 0.80 or 1.00 g of low-gelling-temperature agarose (A-0701 from Sigma) to 1 litre of PBS, and dissolve by autoclaving for 1 hour at 125°C and 20 psi (1.4 bar). Rapidly cool to 4°C to prevent clotting.

Preparation of liver slices started when three to five cores were ready, and was finished within 10-15 minutes after the last core had been prepared. The first slice from each core was discarded. Subsequent slices were collected in slicing buffer (Figure 3.2-b) and kept on ice until further processing. Cores and slices were kept separated from each other.

3.2. TREATMENT OF SLICES

3.2.1. Pre-incubation for 1 hour

Slices were pre-incubated for 1 hour to remove tissue debris, enzymes and other cellular contents released during slice preparation. Some analyses were performed after pre-incubation (treatment group A), but most analyses were carried out only after subsequent treatment (treatment groups B, C, etc).

3.2.1.1. Medium

A synthetic liver culture medium, Williams' medium E (Williams *et al.,* 1971), was used with the addition of certain nutrients and growth factors and an antibiotic/antimycotic solution, according to Table 3.1. Fresh medium was prepared for each experiment and was used for both pre-incubation and, with further additives, for the treatment that followed (for description of treatment see sections 3.2.2 through 3.2.6). The medium was kept refrigerated, and was gassed with 95% O_2 / 5% CO_2 for 10 minutes immediately before use. In order to reduce foaming, the serum was added after the medium had been gassed.

Table 3.1. Composition of fresh medium for pre-incubation

Component	Supplier	Article no.	Qty.	Final concentration
Williams' medium E (with phenol red and $NaHCO_3$, without L-glutamine)	Sigma	W-4128	500 ml	
D-(+)-Glucose monohydrate	Merck	1.04074	550 mg	5.2 mM
1 mM insulin (bovine)‡	Sigma	I-5500	0.5 ml	≈ 1 µM
200 mM L-glutamine	LifeTech	25030-024	5 ml	≈ 2 mM
Antibiotic-Antimycotic (penicillin G, streptomycin sulphate, amphotericin B)	LifeTech	15240-096	5 ml	≈ 100 U/ml, 100 µg/ml, 0.25 µg/ml
FBS (fetal bovine serum)	BioWhit	14-701-F	25 ml	≈ 5% (v/v)

3.2.1.2. Pre-incubation

Each slice was placed on the grid of a titanium roller insert (Roller Insert Type C from Vitron, or type MA-0034 from Alabama) and put in a 20 ml glass scintillation vial (6000348 from Packard) containing 1.70 ml of fresh medium (Figure 3.3). Each vial had a cap (6000239 from Packard) with a 2 mm hole for gas exchange. Vials, inserts, and medium were kept at room temperature.

The process of placing the slices on rollers started when three to five cores had been sliced, and was finished within 10-15 minutes after the last core had been sliced. The slices were placed in the incubator in groups of 10 to 15 (see the following paragraphs). Total time from start of coring until the slices were placed in the incubator ranged from 20-30 minutes to 45-60 minutes in different experiments.

‡ 1 mM insulin: Reconstitute 143.5 mg of anhydrous insulin from bovine pancreas (insulin I-5500 from Sigma) with 25.0 ml of MQ-water. Add some drops of 4 M HCl till a clear solution is achieved. Sterilise through a 0.2 µM filter in portions of 0.5 ml. Keep at ÷20°C for up to 5 years.


a)

b)

Figure 3.3. a) Placing a slice on its roller insert. b) The insert and slice inside a vial

Slices were pre-incubated for 1 hour at 37°C, continuously supplied with 95% O_2 / 5% CO_2 at a flow rate of 0.5 litres/min. The incubator used was a Dynamic Organ Culture Incubator (from Vitron) with an internal chamber rotating at 2 rpm (Figure 3.4). Vials rotated with the chamber at 2 rpm, while inserts rolled inside the vials at approximately 3 rpm providing slices alternately with medium and gas. The chamber could hold at most 96 vials in two depths, but was never used with more than 40.

Figure 3.4. Front view of Dynamic Organ Culture Incubator (Vitron)

The incubator chamber rotates by means of a motor placed in the cabinet behind it. The cabinet also contains a transformer/thermostat unit that heats the chamber. Plastic gas pipes connect the incubator with an external gas source. The rate of gas supply is given by the flow meter attached to the left of the cabinet.

After pre-incubation, some slices were taken as zero-hour controls and analysed (group A). Enzyme leakage was not measured at this stage, because considerable amounts of enzymes had presumably been released in the slicing process and washed out during pre-incubation.

Inserts with remaining slices (treatment groups B and C) were moved to new vials containing 1.70 ml of fresh medium with added solvent (group B) and/or test substance (group C).

The media had been gassed with 95% O_2 / 5% CO_2 for 10 minutes, and vials and media were pre-heated to 37°C. Slices were incubated for 3, 6, or 24 hours in the same incubator and the same conditions as pre-incubation; that is, a temperature of 37°C, gas supply 95% O_2 / 5% CO_2 at 0.5 litres/min, and 2-3 rpm rotation.

3.2.2. Treatment with menadione 200 µM for 3, 6, or 24 hours

Untreated slices were prepared as described in section 3.2.1 (group A).

A stock solution of 20.0 mM menadione§ or dimethylsulfoxide (DMSO) was mixed with fresh medium to obtain the test medium (group C) and control medium (group B), according to Table 3.2. Each medium contained 1% (v/v) DMSO, a concentration that is not considered to affect the analyses (Leeman *et al.,* 1995).

Table 3.2. Composition of media; menadione 200 µM for 3, 6, or 24h

In this table, volumes are given per liver slice, but the media were prepared in larger batches.
For the composition of fresh medium see Table 3.1, page 33.

Slice numbers (treatment group)	fresh medium	20.0 mM menadione	DMSO
A1-A2 (immediately after slicing)	-	-	-
A3-A7 (only pre-incubated)	1.700 ml	-	-
B1-B15 (negative control)	1.683 ml	-	17.0 µl
C1-C15 (menadione 200 µM)	1.683 ml	17.0 µl	-

Subgroups of slices from groups B and C were incubated for 3, 6, or 24 hours according to Table 3.3. After incubation, slices were taken out of their vials and subjected to various analyses (see Table 3.3; analyses are described in section 3.3, page 40 ff). Due to time constraints, the MTT test was not performed in the 0h and 6h groups. Incubation media were put on ice and used for the determination of enzyme leakage (section 3.3.2).

§ 20.0 mM menadione: Dissolve 34.4 mg of menadione (M-5625 from Sigma) in 10.0 ml of DMSO (D-8779 from Sigma). Keep at room temperature and protected from light, for up to 1 week.

Table 3.3. Number of replicates analysed; menadione 200 µM for 3, 6, or 24h

Slice numbers	Time	Slice weights (section 3.3.1)	Enzyme leakage (section 3.3.2)	Remaining enzymes (section 3.3.2.5)	MTT test (section 3.3.3)	Histology (section 3.3.6)
A1-A2	–1h	-	-	-	-	2
A3-A7	0h	all 5	-	3	-	2
B1-B5	3h	all 5	all 5	1	2	2
B6-B10	6h	all 5	all 5	3	-	2
B11-B15	24h	all 5	all 5	1	2	2
C1-C5	3h	all 5	all 5	1	2	2
C6-C10	6h	all 5	all 5	3	-	2
C11-C15	24h	all 5	all 5	1	2	2

3.2.3. Treatment with ferrous sulphate 100 µM for 3, 6, or 24 hours

Untreated slices were prepared as described in section 3.2.1 (group A).

A stock solution of 10.0 mM ferrous sulphate ($FeSO_4$)** or water was mixed with fresh medium to obtain the test medium (group C) and control medium (group B), according to Table 3.4. Each medium contained 1% (v/v) sterile water.

Table 3.4. Composition of media; $FeSO_4$ 100 µM for 3, 6, or 24h

In this table, volumes are given per liver slice, but the media were prepared in larger batches.
For the composition of fresh medium see Table 3.1, page 33.

Slice numbers (treatment group)	fresh medium	10.0 mM $FeSO_4$	sterile water
A1 (immediately after slicing)	-	-	-
A2-A5 (only pre-incubated)	1.700 ml	-	-
B1-B16 (negative control)	1.683 ml	-	17.0 µl
C1-C19 ($FeSO_4$ 100 µM)	1.683 ml	17.0 µl	-

** 10.0 mM ferrous sulphate ($FeSO_4$): Dissolve 55.6 mg of ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) (F-2387 from Sigma) in 20.0 ml of sterile water (82479-E from Braun). Keep cold and protected from light, for up to 1 day.

Slices were incubated for 3, 6, or 24 hours according to Table 3.5 and analysed according to Table 3.5 below and section 3.3, page 40 ff. Incubation media were put on ice and used for the determination of enzyme leakage (section 3.3.2).

Table 3.5. Number of replicates analysed; $FeSO_4$ 100 µM for 3, 6, or 24h

Slice numbers	Time	Slice weights (section 3.3.1)	Enzyme leakage (section 3.3.2)	Remaining enzymes (section 3.3.2.5)	MTT test (section 3.3.3)	Fe and K (sections 3.3.4 and 3.3.5)	Histology (section 3.3.6)
A1	–1h	-	-	-	-	-	1
A2-A5	0h	all 4	-	1	-	2	1
B1-B6	3h	all 6	all 6	1	2	2	1
B7-B10	6h	all 4	all 4	1	-	2	1
B11-B16	24h	all 6	all 6	1	2	2	1
C1-C7	3h	all 7	all 7	1	2	2	2
C8-C12	6h	all 5	all 5	1	-	2	2
C13-C19	24h	all 7	all 7	1	2	2	2

3.2.4. Treatment with 3 concentrations of ferrous sulphate; general aspects

In each of the following experiments, three concentrations of ferrous sulphate were tested; 100-200-400 µM, or 200-1000-5000 µM. (The unit µM is used here to have a common scale for all the $FeSO_4$ culture media.)

Untreated slices were prepared as described in section 3.2.1 (group A). Fresh medium with DMSO was used for negative controls (group B). Menadione 200 µM was used for positive toxic controls (group C).

Stock solutions of 40.0 or 500 mM ferrous sulphate††, or 20.0 mM menadione (described earlier), together with sterile water and/or DMSO, were mixed with fresh medium to obtain

†† 40.0 or 500 mM ferrous sulphate: Dissolve 0.1112 g or 1.390 g, respectively, of $FeSO_4 \cdot 7H_2O$ (F-2387 from Sigma), in 10.0 ml of cold, sterile water (82479-E from Braun). Keep cold and protected from light. Use immediately.

the control media (groups B, C) and test media (groups D, E, F), according to Table 3.7 and Table 3.8. Each medium contained 1% (v/v) DMSO.

After pre-incubation, inserts with remaining slices (groups B through F) were moved to new vials containing 1.70 ml of control medium or test medium. The media had been gassed with 95% O_2 / 5% CO_2 for 10 minutes, and vials and media were pre-heated to 37°C. All slices were incubated for 24 hours in the same incubator and the same conditions as pre-incubation; that is, a temperature of 37°C, gas supply 95% O_2 / 5% CO_2 at 0.5 litres/min, and 2-3 rpm rotation. After treatment, slices were analysed according to Table 3.6 below and section 3.3, page 40 ff. Incubation media were put on ice and used to determine enzyme leakage (section 3.3.2).

Table 3.6. Number of replicates analysed; three concentrations of $FeSO_4$ for 24h

(A, untreated; B, negative control; C, positive control; D, E, F, increasing concentrations of $FeSO_4$.)

Slice numbers	Time	Slice weights (section 3.3.1)	Enzyme leakage (section 3.3.2)	Remaining enzymes (section 3.3.2.5)	MTT test (section 3.3.3)	Fe and K (sections 3.3.4 and 3.3.5)	Histology (section 3.3.6)
A1	–1h	-	-	-	-	-	1
A2-A7	0h	all 6	-	1	2	2	1
B2-B7	24h	all 6	all 6	1	2	2	1
C2-C7	24h	all 6	all 6	1	2	2	1
D1-D7	24h	all 7	all 7	1	2	2	2
E1-E7	24h	all 7	all 7	1	2	2	2
F1-F7	24h	all 7	all 7	1	2	2	2

3.2.5. Treatment with ferrous sulphate 100, 200, or 400 µM for 24 hours

The various slices were treated with culture media according to Table 3.7 and incubated and analysed as described in section 3.2.4 above.

Table 3.7. Composition of media; $FeSO_4$ 100, 200, or 400 µM for 24h

In this table, volumes are given per liver slice, but the media were prepared in larger batches.
For the composition of fresh medium see Table 3.1, page 33.

Slice numbers (treatment group)	fresh medium	40.0 mM $FeSO_4$	20.0 mM menadione	sterile water	DMSO
A1 (after slicing)	-	-	-	-	-
A2-A7 (pre-incubated)	1.700 ml	-	-	-	-
B2-B7 (negative control)	1.666 ml	-	-	17.0 µl	17.0 µl
C2-C7 (positive control)	1.666 ml	-	17.0 µl	17.0 µl	-
D1-D7 ($FeSO_4$ 100 µM)	1.666 ml	4.3 µl	-	12.7 µl	17.0 µl
E1-E7 ($FeSO_4$ 200 µM)	1.666 ml	8.5 µl	-	8.5 µl	17.0 µl
F1-F7 ($FeSO_4$ 400 µM)	1.666 ml	17.0 µl	-	-	17.0 µl

3.2.6. Treatment with ferrous sulphate 200, 1000, or 5000 µM for 24 hours

The various slices were treated with culture media according to Table 3.8 and incubated and analysed as described in section 3.2.4 above.

Table 3.8. Composition of media; $FeSO_4$ 200, 1000, or 5000 µM for 24h

In this table, volumes are given per liver slice, but the media were prepared in larger batches.
For the composition of fresh medium see Table 3.1, page 33.

Slice numbers (treatment group)	fresh medium	500 mM $FeSO_4$	20.0 mM menadione	sterile water	DMSO
A1 (after slicing)	-	-	-	-	-
A2-A7 (pre-incubated)	1.700 ml	-	-	-	-
B2-B7 (negative control)	1.666 ml	-	-	17.0 µl	17.0 µl
C2-C7 (positive control)	1.666 ml	-	17.0 µl	17.0 µl	-
D1-D7 ($FeSO_4$ 200 µM)	1.666 ml	0.68 µl	-	16.3 µl	17.0 µl
E1-E7 ($FeSO_4$ 1000 µM)	1.666 ml	3.4 µl	-	13.6 µl	17.0 µl
F1-F7 ($FeSO_4$ 5000 µM)	1.666 ml	17.0 µl	-	-	17.0 µl

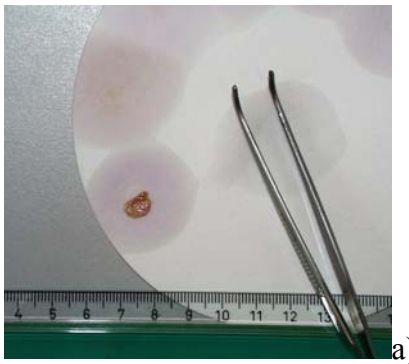
3.3. ANALYSES

3.3.1. Measuring slice weights

The weights of all slices, except those taken for histology immediately after slicing, were recorded to be able to adjust certain other results according to slice weights.

Using a pair of tweezers, each slice was dried gently on filter paper (331511 / 520B from Schleicher) that had been moistened with a few drops of the slice's own incubation medium (Figure 3.5-a), and then weighed on an analytical balance (AT261 DeltaRange from Mettler). The weighing was performed directly in the vial containing the appropriate reagent or solution for further analyses, such as 1% triton for determination of remaining enzymes (section 3.3.2.5) or 10% formalin for histology (section 3.3.6). The tweezers were rinsed in cold PBS between handling every slice.

Williams' medium E contains only trace amounts of iron (0.000025 μM, or 0.0001 mg/l, $Fe(NO_3)_3 \cdot 9H_2O$; Williams *et al.*, 1971), but varying amounts of ferrous sulphate had been added before treatment. To minimise the influence of Fe in medium on the following iron assay (section 3.3.4), slices assigned for this analysis were rinsed in cold PBS (Figure 3.5-b) prior to drying and weighing, and the filter paper was moistened with PBS instead of incubation medium.


a)

b)

Figure 3.5. a) Drying a slice on filter paper (scale: cm, mm). b) Rinsing a slice in PBS

3.3.2. Enzyme leakage

Incubation media (Figure 3.6-a) were kept on ice and then centrifuged at ≈2400 xg (5000 rpm; radius 85 mm) for 6 minutes at 4°C. Supernatants (Figure 3.6-b) were diluted 5-fold with fresh medium (100 µl supernatant + 400 µl medium) to bring enzymes within ranges of measurement. The dilution was performed in 1 ml vials (73.910.004 / Cobas sample cups from Sarstedt) designed to fit the analytic instrument. Vials were stored at ÷70°C awaiting analyses, which were performed within one week after treatment. (Preliminary experiments had indicated that enzyme activities were satisfactorily preserved by ultra-freezing for the period of interest; data not shown). Enzyme activities were measured on a Cobas Fara II automatic analyser (from Roche), using reagent kits specific to the individual enzymes.

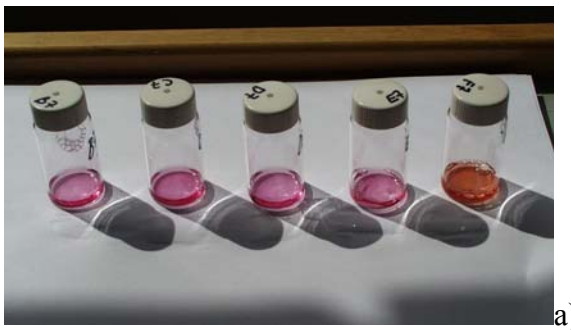
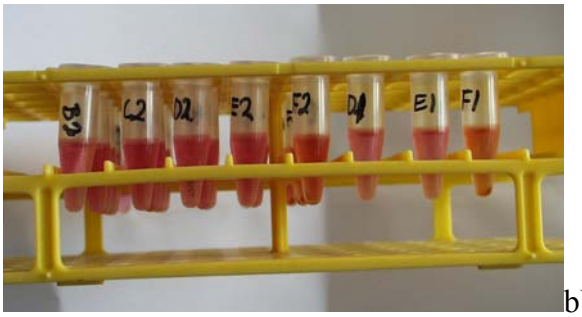

a)

b)

Figure 3.6. a) Incubation media after removal of slices. b) Centrifuged media

Note the pellets of cellular debris at the centrifuge tube bottoms.

3.3.2.1. Alanine aminotransferase (ALAT)

In this study ALAT activity was measured using a UV-kinetic reagent kit (07 3638 4 / Unimate 3 ALAT from Roche), based on recommendations by the SFBC (1982a) and IFCC (1986a). The kit contained i.a. L-alanine, α-ketoglutarate, NADH, and LDH.

ALAT catalyses the physiological reaction:

$$\text{L-alanine} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{ALAT}} \text{L-glutamate} + \text{pyruvate}$$

This reaction forms the principle for the quantitative analysis of the enzyme. Biological samples containing ALAT are mixed with reagent kits holding reagents for both the above reaction and a second one in which NADH is oxidised to NAD^+, catalysed by the enzyme lactate dehydrogenase (LDH):

$$\text{pyruvate} + \text{NADH} + H^+ \xrightleftharpoons{\text{LDH}} \text{L-lactate} + NAD^+$$

NADH and NAD^+ are the reduced and oxidised forms, respectively, of the enzyme cofactor, nicotinamide adenine dinucleotide. The rate of NADH oxidation, or disappearance, is directly related to the ALAT activity from the first reaction. NADH levels are measured photometrically at 340 nm by the Cobas Fara II analyser (from Roche).

3.3.2.2. Aspartate aminotransferase (ASAT)

In this study ASAT activity was measured using a UV-kinetic reagent kit (07 3641 4 / Unimate 3 ASAT from Roche), based on recommendations by the SFBC (1982b) and IFCC (1986b). The kit contained i.a. L-aspartate, α-ketoglutarate, NADH, and MDH.

ASAT catalyses the physiological reaction:

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{ASAT}} \text{L-glutamate} + \text{oxaloacetate}$$

Oxaloacetate is involved in a second reaction where NADH is oxidised, catalysed by the enzyme malate dehydrogenase (MDH):

$$\text{oxaloacetate} + \text{NADH} + H^+ \xrightleftharpoons{\text{MDH}} \text{L-lactate} + NAD^+$$

NADH levels, indicating ASAT activity, are measured photometrically at 340 nm.

3.3.2.3. Glutamate dehydrogenase (GLDH)

In this study GLDH activity was measured using a UV-kinetic reagent kit (1929992 from Roche), based on recommendations by the DGKC (1972). The kit contained i.a. α-keto-glutarate, NADH, and NH_4^+ (ammonium).

GLDH catalyses the physiological reaction:

$$\alpha\text{-ketoglutarate} + NADH + NH_4^+ \xrightleftharpoons{GLDH} \text{L-glutamate} + NAD^+$$

Here, NADH is oxidised directly in the GLDH dependent reaction. NADH levels are measured photometrically at 340 nm.

3.3.2.4. Lactate dehydrogenase (LDH)

In this study LDH activity was measured using a UV-kinetic reagent kit (07 3657 0 / Unimate 3 LDH SFBC from Roche), based on recommendations by the SFBC (1982c). The kit contained i.a. pyruvate and NADH.

LDH catalyses the physiological reaction:

$$\text{pyruvate} + NADH + H^+ \xrightleftharpoons{LDH} \text{L-lactate} + NAD^+$$

This is identical with the second reaction of the ALAT assay (see above). NADH levels, indicating LDH activity, are measured photometrically at 340 nm.

3.3.2.5. Remaining enzymes in slices

To calculate the relative amounts of enzymes that had leaked out during treatment, the remaining enzymes in slices were estimated. This was accomplished by lysing slices with a surfactant, triton, and measuring the activities of the four enzymes (ALAT, ASAT, GLDH, and LDH) in the resulting lysate.

One or three slices per treatment group were treated individually in polypropylene tubes (368632 / CryoTubes from Nunc) with 1.50 ml of a 1% triton solution‡‡ at room temperature overnight on a rocking table (SM1 from Desaga). The lysate was centrifuged at ≈2000 xg (3000 rpm; radius 205 mm) for 10 minutes at room temperature. Supernatants were diluted 20-fold with fresh medium (25.0 µl supernatant + 475 µl medium) in Cobas vials. The vials were stored at ÷70°C for up to one week awaiting analyses. Enzyme activities were measured using a Cobas Fara II automatic analyser and reagent kits as described above for incubation media.

3.3.3. MTT test

The amount of formazan (blue) produced by cells when incubated with MTT (yellow) is an indication of mitochondrial function and cell viability (section 2.4.4, page 27).

Two slices per group (except from the 0h and 6h groups in the 3/6/24 hour experiments) were incubated individually in polypropylene tubes with 1.50 ml of a 1.21 mM MTT solution§§ at 37°C in the dark for 40 minutes on a rocking table (SM1 from Desaga). The slices were rinsed in cold PBS, and formazan was extracted from the slices in polypropylene tubes with 1.50 ml of isopropanol (article 1004858 from Arcus) at room temperature in the dark overnight on a rocking table. Slices were then removed from the extracts, which were kept refrigerated for 2-4 days before they were analysed.

200 µl of extract was pipetted in two parallels per slice onto a flat-bottomed 96-well microplate (439454 / MaxiSorp F96 from Nunc). The absorbency, or optical density, at 570 nm (OD_{570}) was measured using an automatic plate reader (MRX from Dynatech) with the appropriate software (BioLinx from Dynatech). Pure isopropanol was used as background.

‡‡ 1% triton: Dilute 1.00 ml of triton X-100 (article T-6878 from Sigma) with 99 ml of sterile water (82479-E from Braun). Keep at room temperature for up to 6 months.

§§ 1.21 mM (0.50 mg/ml) MTT: Dissolve 50 mg of MTT (M-2128 from Sigma) in 100 ml of PBS (for the composition of PBS see footnote in section 3.1, page 30). Keep at room temperature and protected from light, for up to 1 day. Filter through filter paper (10331514 / 520B½ from Schleicher) immediately before each use.

3.3.4. Analysis of iron content

Effects of treatment could be related not only to iron concentrations in media, but to the degree of passive and/or active iron uptake during treatment and consequently to iron contents in tissue. Therefore, in the experiments involving iron, two slices from each treatment group (including controls) were analysed for iron content using inductively coupled plasma atomic emission spectrometry (ICP-AES). The same slices were also analysed for potassium content.

3.3.4.1. Sample preparation

To minimise the influence of Fe in medium on the following iron assay, slices assigned for this analysis were rinsed in cold PBS (Figure 3.5-b, page 41) prior to drying and weighing, and the filter paper was moistened with PBS instead of incubation medium.

Each slice was put in a 1 ml polypropylene tube (366656 / CryoTubes from Nunc) containing 500 µl of sterile water (82479-E from Braun). Slice weights were recorded (for details on the weighing procedure see section 3.3.1). The slices were kept at ÷70°C until further processing and analyses.

After thawing, each slice was transferred to an acid-washed, 15 ml polypropylene tube (62.554 from Sarstedt) together with its 0.5 ml of water. 1 ml of concentrated nitric acid (65% HNO_3 from ChemScan) was used to rinse the smaller tube and was then transferred to the larger tube to dissolve organic components. 0.5 ml of concentrated hydrochloric acid (30% HCl from ChemScan) was added to solubilise the iron. The resulting mixture was incubated at 70°C overnight. Using an electronic auto-dispenser (130100 / Microlab1000 from Hamilton), 500 µl of a 10 ppm scandium (Sc) solution*** was added as internal standard. Before assaying, the sample was diluted to approximately 5 ml with MQ-water, so the matrix was ≈20% nitric acid, ≈10% hydrochloric acid, and ≈1 ppm Sc. Since 90-95% of the slices used for iron analyses weighed less than 30 mg (data not shown), cellular material generally comprised less than 0.6% of the 5 ml solution.

*** 10 ppm Sc: Use only acid-washed glassware. Pipette 5.00 ml of 1000±3 ppm Sc (Spectrascan 8055 from Teknolab) into a 500 ml flask. Fill with MQ-water.

Reference samples were prepared from aliquots of approximately 50 mg dried, pulverised bovine liver (SRM 1577b from NIST) with specified contents of iron (Fe), potassium (K), and certain other elements. Each aliquot was suspended in 500 µl of MQ-water. To imitate treatment of slices during experiments, every third reference sample was spiked with 250 µl of a 20 mM ferrous sulphate solution††† corresponding to 112 ppm Fe. Another third was spiked with 250 µl of a 100 ppm Fe standard solution‡‡‡. (Both versions of spiking would add ≈5 ppm Fe to the prepared sample.)

For blank samples 500 µl of MQ-water was used. All blank samples and reference samples were then treated in the same way as the liver slices; 1 ml nitric acid, 0.5 ml hydrochloric acid, incubated at 70°C overnight, added 500 µl of 10 ppm Sc, diluted to 5 ml with MQ-water.

3.3.4.2. Analysis by ICP-AES

An analysis program was set up on an electronic ICP-AES reader (Optima 3300 DV from PerkinE) with the appropriate software, and was run twice with 50 samples, 6 blank samples, and 9 reference samples in each run. The program included five standard solutions of 0-20 ppm Fe and K§§§, to which the instrument was calibrated before each run and between samples. Blanks and references were divided in three groups and placed before, in the middle, and after the other samples. All samples, blanks, references and standard solutions contained 1 ppm Sc as internal standard.

---

††† 20 mM ferrous sulphate: Weigh out 556.0 mg of $FeSO_4 \cdot 7H_2O$ (F-2387 from Sigma) and transfer quantitatively to a 100 ml flask. Dissolve in ≈50 ml of MQ-water, add 10 ml of concentrated hydrochloric acid (30% HCl from ChemScan), and allow for temperature equalisation. Fill to 100 ml with MQ-water to obtain an intermediate of 200 mM Fe. Pipette 10.0 ml of this solution into a new 100 ml flask and fill with MQ-water. Corresponds to 112 ppm Fe.

‡‡‡ 100 ppm Fe standard: Pipette 10.0 ml of 1000±3 ppm Fe (Spectrascan 8004 from Teknolab) into a 100 ml flask and fill with MQ-water.

§§§ Standard solutions of 20, 5.0, 1.0, 0.50, and 0.00 ppm Fe and K: Use acid-washed full pipettes and 100 ml flasks. Prepare intermediate solutions of 100 and 10 ppm Fe and 100 and 10 ppm K using 1000±3 ppm Fe and 1000±3 ppm K (Spectrascan 8004 and 8007 from Teknolab), and MQ-water. Prepare the standards from these intermediates and MQ-water, with the addition of concentrated nitric and hydrochloric acids (from Chemscan), and 10 ppm Sc (see separate footnote for Sc), so that the final standards have a matrix of ≈20% nitric acid, ≈10% hydrochloric acid, and 1 ppm Sc.

Iron (Fe) was detected at 259.939 nm, potassium (K) at 766.490 nm, and scandium (Sc) at 424.683 nm. The blank samples were used to calculate zero levels, detection limits (DL), and quantification limits (QL).

The measured Fe concentrations (result: µg/ml) were adjusted for slice weights (result: µg/mg) and reported as percentages relative to the negative controls at 0 hours.

3.3.5. Analysis of potassium content

Potassium was measured simultaneously with iron, using the same samples and methods as described in section 3.3.4.

3.3.6. Histology

3.3.6.1. Tissue fixation, sectioning, and staining

One or two slices from each treatment group, in addition to slices taken immediately after slicing, were stored individually in 10 ml of 10% NBF (neutral-buffered formalin)****. After fixation, slices were infiltrated with paraffin wax (VO-5-11050 from Vogel) by a vacuum infiltration processor (VIP-E150B / Tissue-Tek from Sakura) and embedded in blocks of paraffin wax. Slices were sectioned at a nominal thickness of 3-5 µm using a rotation microtome (RM2155 from Leica), and the sections were mounted on object slides. The slides were heated to 60°C for 1 hour in order to remove most of the wax, before they were stained with hematoxylin and eosin by an automatic slide stainer (DRS-60 from Sakura) (for details on the staining process see appendix A–3). Finally, the stained sections were fixed in pertex glue (00801 from Histolab) under cover slips.

**** 10% NBF: Dissolve 8.90 g (=50.0 mmol) $Na_2HPO_4 \cdot 2H_2O$ (1.06580 from Merck) and 4.14 g (=30.0 mmol) $NaH_2PO_4 \cdot H_2O$ (1.06346 from Merck) in approximately 0.5 litres of RO-water. Add 100 ml formalin (1.03999 from Merck) and fill with RO-water to 1.00 litre; pH 6.9. (Formalin is a solution of 37% (w/v) formaldehyde gas in water with 10-15% methanol, so 10% NBF contains less than 4% formaldehyde.)

3.3.6.2. Morphological description

The morphology of the tissue sections was assessed qualitatively with respect to tissue damage using a microscope. Organelle degradation, nuclear shrinkage, and tissue necrosis were estimated. Samples from each treatment group were compared to negative controls and positive controls from the same experiment.

3.4. STATISTICAL METHODS

The number of treatment groups was 6 or 7 in each experiment. The number of replicates in each group, that is, the number of liver slices or incubation media analysed the same way, ranged from 2 (MTT test) to 7 (slice weights and enzyme leakage). This was based partly on practical restraints and partly on measures of variation obtained in preliminary studies (data not shown). Thus, from each analysis of a treatment group, the sample mean of 2 to 7 liver slices or incubation media was used for further calculations.

In three of the four main study designs, 2 identical experiments were performed (n=2), using 1 animal per experiment. In the fourth design, 4 experiments were performed (n=4). However, several treatment groups were the same in various experiments, and data from analogue groups could be pooled for every parameter to achieve up to 10 sample means (n=2 to 10), each representing 2 to 7 liver slices. A higher n allows greater power of hypothesis testing, i.e. greater possibility of detecting statistically significant differences between treatment groups.

Excel 97 (from Microsoft Co.) was used to calculate the number of replicates (number of slices), the sample mean, and the standard deviation (SD) for each parameter in all treatment groups and all experiments, and to present the results graphically. SigmaStat 2.0 for Windows (from Jandel Co.) was used to calculate the number of experiments (n, number of animals), the average of sample means, and the standard error of the mean (SE) for each parameter in the pooled data, and to perform the following statistical analyses:

All data sets were assumed to belong to a normal distribution, and the variances of different data sets were assumed to be homogeneous. Thus, parametric analyses were performed. To detect effects of treatment, a one-way analysis of variance (ANOVA) was executed for each parameter observed at each observation time (3, 6, or 24 hours), comparing different

treatment groups at each time point. To detect any effects of the length of treatment, ANOVA was also carried out with different observation times of a single treatment, e.g. only negative control or only $FeSO_4$ 100 μM. Significant ANOVA results ($p < 0.05$) were followed by post-hoc multiple comparison tests between control group and other groups, using Dunnett's t test to identify significant differences ($p < 0.05$).

To check the above assumptions of normality and homogeneity, data sets comprising at least 3 sample means were assessed for normality using the Kolmogorov-Smirnov test with Lilliefors' correction, and for homogeneity of variance using the Levene median test. (These methods do not apply to data sets with less than 3 observations.) Positive results from both the normality test ($p \geq 0.05$) and the variance test ($p \geq 0.05$) supported the choice of parametric analyses, whereas negative results from either test suggested non-parametric analyses.

Although the data were still assumed to be normal and homogeneous, additional non-parametric analyses were performed for these data sets, and any discrepancies between the parametric and non-parametric analyses were noted. Kruskal-Wallis ANOVA on ranks was executed for each parameter and observation time to identify effects of treatment, and for different time points of a single treatment to identify effects of the length of treatment. Significant ANOVA results ($p < 0.05$) were followed by post-hoc multiple comparisons using Dunn's rank sum test to identify significant differences ($p < 0.05$).

4. RESULTS AND DISCUSSION

4.1. PRELIMINARY RESULTS

4.1.1. Method development

In total 16 experiments were performed.

The first three experiments were aimed at operator training and method development, e.g. the testing of a new incubator and solving problems regarding the preparation of tissue cores from the small rat livers.

The operation of equipment and performance of experiments was relatively straightforward, but time consuming. Each experiment required 2 persons for approximately 1 full day to prepare and incubate the rat liver slices and carry out analyses immediately after incubation (slice weights, MTT test, enzyme leakage), plus many hours of follow-up analyses over a period of weeks (MTT test, enzyme leakage, Fe and K content, and histology).

The first experiments were largely exploratory, and quantitative results are not presented. However, these experiments involved different concentrations of menadione and were used to pick the concentration of menadione for positive controls in later experiments (200 μM) and also formed the basis for the methods and materials presented in chapter 3.

In the first three experiments the animals were fed normally until study start, and microscopic analysis of the rat liver slices showed large numbers of white starch crystals inside the cells. These crystals impaired visual observation of the cells in histology, and in all subsequent studies the animals were starved for the last part of the last night to eliminate or reduce the amount of starch crystals.

Results from 13 experiments indicate that an exsanguinous, starved rat liver should be 8 grams or more in order to get 50 good slices of 8 mm diameter and 250 μm thickness. The exsanguinous liver constituted approximately 3.5% of the starved body weight, which must therefore be 230 grams at study start, and the normal body weight the previous day must be 250 grams (see appendix A–2).

4.1.2. Histology results

Histological analysis from the first three experiments showed extensive tissue damage in all samples. This may be due to treatment or sample preparation. The quality of the histological samples did not improve through further experiments. Therefore, the histological analysis was not completed, and histology results are not presented.

4.1.3. Excluded experiments

As described above, quantitative results from the first three experiments are not presented.

Another two experiments are excluded because of divergent study parameters (wrong method of sacrifice, and wrong composition of test medium).

One experiment is excluded because the rat was female instead of male. As mentioned in section 3.1.1 (page 29), only male Sprague-Dawley rats should be used, and they should be between 6 and 8 weeks old at study start. By mistake a female rat aged 8 ½ weeks was used in one experiment, and the observed parameters in that experiment (data not shown) diverged considerably from those obtained with male rats. The female rat did not gain any weight during acclimatisation (see appendix A–2), which suggests that its lifetime growth had flattened off, the individual had reached adulthood, and its physiological conditions were probably not comparable to the other animals with respect to the parameters of interest.
It may be difficult to establish whether the sex as such or the state of growth was decisive in this case, but the ages and weights of subsequent animals were examined with extra scrutiny.

4.2. PRESENTATION OF RESULTS

Results from 10 experiments are reported henceforth. For raw data see appendix A–5.

The results are generally presented by graphic charts rather than tables or line-listings. The graphs display the results as averages of sample means, with error bars representing ±SE. Chapters 4.3 through 4.6 describe the findings from 4 different study designs, and the appropriate graphs are placed in appendix A–4.1 through A–4.4. Chapter 4.7 compares all experiments collectively by parameters observed, and the graphs for this are given in appendix A–4.5.

The numbers of experiments (n=2 to 10) and replicates (2 to 7) are given for each data set. Significant differences between treatment groups or treatment times are indicated by the following symbols above the respective chart bars, single symbols meaning $p < 0.05$ and double symbols meaning $p < 0.01$:

- * or ** significantly different from negative control (* $p<0.05$, ** $p<0.01$) (applies to positive control vs. neg. ctrl., and $FeSO_4$ vs. neg. ctrl.);
- # or ## significantly different from earliest observation of the same treatment (# $p<0.05$, ## $p<0.01$) (applies to neg. ctrl. 24h vs. 0h, or pos. ctrl. 24h vs. 3h, or $FeSO_4$ 100 µM 24h vs. 3h);
- ¢ or ¢¢ significantly different from lowest concentration of same substance (¢ $p<0.05$, ¢¢ $p<0.01$) (5000 and 1000 µM of $FeSO_4$ vs. lover $FeSO_4$).

A treatment group may satisfy more than one of these conditions, indicated by more than one symbol at the respective bar (e.g., * and #).

4.3. RESULTS FROM TREATMENT WITH MENADIONE 200 µM FOR 3, 6, OR 24 HOURS

Two experiments (n=2) were performed with 7 treatment groups; 0, 3, 6, and 24 hours negative control and 3, 6, and 24 hours positive control (menadione). For the number of replicates (slices or media) see Table 3.3, page 36.

Six quantitative parameters were recorded:

1) Slice weights (mg),
2) MTT test in the 3h and 24h groups (% of 24h negative control),
3) ALAT leakage (% of total ALAT),
4) ASAT leakage (% of total ASAT),
5) GLDH leakage (% of total GLDH), and
6) LDH leakage (% of total LDH)

In the MTT test the actual variable measured was optical density at 570 nm (OD_{570}), which was adjusted for slice weight and reported as percentages of 24h negative control. The MTT test was not performed in the 0h and 6h groups.

In the enzyme assays the measured variables were enzyme activities (U/l) in culture medium. The activities were reported as percentages of the sum of activity in medium and remaining enzymes in slices. Enzyme leakage at 0h could not be determined.

The results from the 2 experiments are presented graphically in appendix A–4.1, page 77 ff. The following significant differences were found:

Table 4.1. Significant results from experiments with menadione 200 µM

Assay	Lower assay	Higher assay	p	Cause
Slice weights	neg. controls, 24h	pos. controls, 24h	$p < 0.05$	Menadione treatment
MTT results	pos. controls, 24h	neg. controls, 24h	$p < 0.01$	Menadione treatment
ASAT leakage	pos. controls, 3h	pos. ctrl., 6h, 24h	$p < 0.01$	Incubation time
LDH leakage	pos. controls, 3h	pos. ctrl., 6h, 24h	$p < 0.05$	Incubation time
LDH leakage	neg. controls, 6h	pos. controls, 6h	$p < 0.01$	Menadione treatment

The slice weights increased with menadione treatment. Many of the slices treated with menadione were visibly harder than the others after treatment. This may indicate an

encapsulation of the outer cells as they reacted to the added toxin, and an apparent resistance to subsequent mechanical stress.

Slice viability (MTT results) decreased with menadione treatment, and LDH leakage increased with menadione treatment. In slices treated with menadione the leakage of ASAT and LDH increased with longer treatment time.

Leeman *et al.* (1995), using an oxygen level of 40%, found that MTT results were significantly lower in rat liver slices treated for 4 hours with 50, 100, 200 or 400 µM menadione than in negative controls. They also found that LDH leakage was significantly higher in slices treated with 100, 200 or 400 µM menadione than in negative controls.

Chan *et al.* (1992) reported cytotoxic effects in rat liver slices treated with 100-300 µM menadione with 95% oxygen. Wright and Paine (1992) reported cytotoxic effects of 200 µM menadione only in isolated hepatocytes and not in liver slices with 21% oxygen. This was explained by low absorption of menadione in the inner cells of liver slices or low levels of oxygen in the inner cells of slices compared to isolated cells.

In the present experiments, several assays confirmed the expected cytotoxic effects of menadione on rat liver slices. The culture media, incubation system, gas supply (95% O_2 / 5% CO_2) and analyses were sufficient to demonstrate the toxicity of menadione compared to negative controls. This model may probably be used in hepatotoxicological studies on other substances.

4.4. RESULTS FROM TREATMENT WITH FERROUS SULPHATE 100 µM FOR 3, 6, OR 24 HOURS

Two experiments (n=2) were performed with 7 treatment groups; 0, 3, 6, and 24 hours negative control and 3, 6, and 24 hours $FeSO_4$ 100 µM. There was no positive control group (menadione) in these experiments. For the number of replicates see Table 3.5, page 37.

Six parameters were recorded as described in the previous section. In addition the contents of potassium and iron were measured, increasing the number of parameters to 8:

7) Potassium (K) content (% of 0h negative control), and
8) Iron (Fe) content (% of 0h negative control)

In these assays the variables measured were metal concentrations (ppm, or µg/ml) in sample after dissolution of slice. The concentrations were adjusted for slice weight and reported as percentages of 0h negative control.

The results from the 2 experiments are presented graphically in appendix A–4.2, page 80 ff. The following significant differences were found:

Table 4.2. Significant results from experiments with $FeSO_4$ 100 µM

Assay	Lower assay	Higher assay	p	Cause
MTT results	$FeSO_4$ 100 µM, 24h	$FeSO_4$ 100 µM, 3h	$p < 0.05$	Incubation time
MTT results	$FeSO_4$ 100 µM, 24h	neg. controls, 24h	$p < 0.05$	$FeSO_4$ treatment
GLDH leak.	neg. controls, 0h	neg. controls, 6h, 24h	$p < 0.05$	Incubation time
K content	neg. controls, 0h	neg. ctrl., 3h, 6h, 24h	$p < 0.05$	Incubation time

MTT results decreased with time and with iron treatment. GLDH leakage increased with time, but no differences were evident between iron-treated slices and negative controls with regard to GLDH leakage.

The K content in slices increased significantly from 0 hours to 3 hours in negative controls. Increased K content during the first hours of incubation may be caused by potassium in the culture medium (the medium contained the buffer $NaHPO_4$ / KH_2PO_4 plus KCl) or by a transitory potassium leakage during slice preparation and subsequent recovery. If the latter is the case, the recovery must take longer time than the one hour of pre-incubation but is probably complete within 3 to 6 hours, according to the peak in K content at 6 hours.

Studies on *in vitro* treatment of rat liver slices with iron (II) sulphate were not found in literature, so the results of the present study are not directly comparable to others. However, several studies have shown that iron (II) sulphate causes lipid peroxidation in rat liver homogenate (Pushpendran *et al.*, 1998), isolated rat liver mitochondria (Pushpendran *et al.*, 1998; Bacon *et al.*, 1986), and isolated rat liver lysosomes (Mak and Weglicki, 1985). Lipid peroxidation can disrupt cellular integrity and mitochondrial function, which may then result in enzyme leakage and reduced MTT reduction capability.

The reduction in MTT results with iron treatment can be explained by lipid peroxidation. The lack of other differences between $FeSO_4$ 100 µM and negative controls may be due to this concentration of iron being too low to cause severe cytotoxicity in rat liver slices.

An indication of the lack of effect is the almost identical Fe content in slices treated with $FeSO_4$ 100 µM and negative controls (see the last graph in appendix A–4.2).

4.5. RESULTS FROM TREATMENT WITH FERROUS SULPHATE 100, 200, OR 400 µM FOR 24 HOURS

Two experiments (n=2) were performed with 6 treatment groups; 0 and 24 hours negative control, 24 hours positive control (menadione), and 24 hours $FeSO_4$ 100, 200, or 400 µM. For the number of replicates see Table 3.6, page 38.

The same eight parameters were recorded (see sections 4.3 and 4.4), but now the MTT test was performed in all groups.

The results from the 2 experiments are presented graphically in appendix A–4.3, page 84 ff. The following significant differences were found:

Table 4.3. Significant results from experiments with $FeSO_4$ 100, 200, or 400 µM

Assay	Lower assay	Higher assay	p	Cause
MTT results	pos. controls, 24h	neg. controls, 24h	$p < 0.01$	Menadione treatment
LDH leakage	neg. controls, 24h	pos. controls, 24h	$p < 0.05$	Menadione treatment
K content	pos. controls, 24h	neg. controls, 24h	$p < 0.05$	Menadione treatment

MTT results decreased and LDH leakage increased with menadione treatment (positive control). These results are similar to those obtained in earlier experiments (section 4.3, page 53) and can be explained by mitochondrial dysfunction and cellular disruption.

The K content also decreased with menadione treatment. This may be due to cell membrane dysfunction reducing the influx of K, or cellular disruption causing leakage of K.
The reduced K content may also be caused by mitochondrial dysfunction that leads to reduced ATP levels and less energy available to the Na/K-ATPase pumping K into the cell (Vickers, 1997).

No differences were evident between slices treated with the three concentrations of iron, or between iron treatment and negative controls. The Fe content was almost identical in negative controls and slices treated with $FeSO_4$ 100, 200 or 400 µM (last graph in appendix

A–4.3). These iron concentrations are apparently not sufficient to cause measurable toxic effects in rat liver slices with this study design.

4.6. RESULTS FROM TREATMENT WITH FERROUS SULPHATE 200, 1000, OR 5000 µM FOR 24 HOURS

Four experiments (n=4) were performed with 6 treatment groups; 0 and 24 hours negative control, 24 hours positive control (menadione), and 24 hours $FeSO_4$ 200, 1000, or 5000 µM. For the number of replicates see Table 3.6, page 38.

The same eight parameters were recorded. The MTT test was performed in all groups.

In the category negative controls at 24h, one of four sample means from GLDH leakage was excluded because of extreme deviation from the rest. The GLDH leakage in question was 28.6%, which was ten times higher than the average of the others in the range 1.8% to 4.5%. The high leakage percent was due to a very low content of remaining GLDH in the reference slice in that particular experiment compared to the other experiments, but the reason for the low reference value is not clear. By excluding the one value, the average was changed from 5.5±8.2 % to 2.9±1.0 % (mean ±SE).

The results from the 4 experiments are presented graphically in appendix A–4.4, page 88 ff. The following significant differences were found:

Table 4.4. Significant results from experiments with $FeSO_4$ 200, 1000, or 5000 µM

Assay	Lower assay	Higher assay	p	Cause
Slice weights	neg. controls, 24h	neg. controls, 0h	$p < 0.01$	Incubation time
MTT results	pos. controls, 24h	neg. controls, 24h	$p < 0.01$	Menadione treatment
MTT results	$FeSO_4$ 5000 µM, 24h	neg. controls, 24h	$p < 0.05$	$FeSO_4$ treatment
ASAT leakage	neg. controls, 24h	$FeSO_4$ 5000 µM, 24h	$p < 0.01$	$FeSO_4$ treatment
ASAT leakage	$FeSO_4$ 200 µM, 24h	$FeSO_4$ 5000 µM, 24h	$p < 0.05$	$FeSO_4$ treatment
ASAT leakage	$FeSO_4$ 200 µM, 24h	$FeSO_4$ 1000 µM, 24h	$p < 0.05$	$FeSO_4$ treatment
GLDH leakage	neg. controls, 24h	$FeSO_4$ 5000 µM, 24h	$p < 0.01$	$FeSO_4$ treatment
K content	neg. controls, 0h	neg. controls, 24h	$p < 0.05$	Incubation time

Assay	Lower assay	Higher assay	p	Cause
Fe content	neg. controls, 24h	$FeSO_4$ 5000 µM, 24h	$p < 0.01$	$FeSO_4$ treatment
Fe content	$FeSO_4$ 200 µM, 24h	$FeSO_4$ 5000 µM, 24h	$p < 0.05$	$FeSO_4$ treatment

Note: Concerning GLDH leakage, the difference between $FeSO_4$ 5000 µM at 24h and negative controls at 24h was found using parametric analyses (ANOVA, t test). However, the GLDH results in the $FeSO_4$ 5000 µM group failed the test of normality ($p < 0.01$), and so additional non-parametric analyses were performed (rank-ANOVA, rank sum test) and showed no significant differences with respect to GLDH leakage ($p \geq 0.05$).

Slice weights and K content decreased with treatment time. Leakage of ASAT and GLDH increased with high iron concentrations in the medium (1000-5000 µM). Slice viability (MTT results) decreased with menadione treatment and with the highest iron concentration.

The effect of high $FeSO_4$ concentrations on enzyme leakage was more pronounced than that of lower $FeSO_4$ concentrations both here and in earlier experiments (sections 4.4 and 4.5). As could be expected, the Fe content in slices increased with high Fe concentrations in the medium. It is not certain if the cells had actually absorbed all this iron or if it was only adsorbed to the slice surfaces, but to minimise the influence of incubation media on the iron assay, slices assigned for this analysis had been rinsed in cold PBS prior to drying and weighing (Figure 3.5-b, page 41), and the filter paper used for drying had been moistened with PBS instead of incubation medium (see sections 3.3.1 p. 40, and 3.3.4 p. 45). It is assumed that most of the non-absorbed iron was removed during rinsing and drying, and that the measured biochemical effects were due to iron absorbed by cells.

Iron (II) sulphate causes lipid peroxidation in rat liver tissue (Bacon *et al.*, 1986; Mak and Weglicki, 1985; Pushpendran *et al.*, 1998). Lipid peroxidation can disrupt cellular integrity and mitochondrial function, and this may explain the increased enzyme leakage (ASAT, GLDH) and the reduced MTT results with iron treatment.

4.7. POOLED DATA FROM DIFFERENT STUDY DESIGNS

In order to identify trends that may not be demonstrable in each experiment, data from groups that had received the same treatment in different experiments were pooled and analysed together. This also made it possible to compare treatments that had not been part of the same experiment. Results were compared from up to 10 animals (n=2 to 10; see Table 4.5), each representing 2 to 7 slices in the various treatments and analyses performed.

The results are presented graphically in appendix A–4.5, page 92 ff.

Table 4.5. Number of animals compared in the pooled data from various treatments and analyses

Group	Time	n (slice weights)	n (enzyme leakage)	n (MTT test)	n (K and Fe)
Negative control	0h	10	–	6	8
Negative control	3h	4	4	4	2
Negative control	6h	4	4	–	2
Negative control	24h	10	10	10	8
Positive control	3h	2	2	2	–
Positive control	6h	2	2	–	–
Positive control	24h	8	8	8	6
$FeSO_4$ 100 µM	3h	2	2	2	2
$FeSO_4$ 100 µM	6h	2	2	–	2
$FeSO_4$ 100 µM	24h	4	4	4	4
$FeSO_4$ 100 µM	24h	4	4	4	4
$FeSO_4$ 200 µM	24h	6	6	6	6
$FeSO_4$ 400 µM	24h	2	2	2	2
$FeSO_4$ 1000 µM	24h	4	4	4	4
$FeSO_4$ 5000 µM	24h	4	4	4	4

Because the $FeSO_4$ 100 µM 24h category was part of time-effect studies as well as concentration-effect studies, that category has got two lines in Table 4.5, and it is represented by two identical but differently coloured bars in the pooled charts.

4.7.1. Slice weights

Average slice weights ranged from 19.2 mg in negative controls at 24h, to 26.1 mg in positive controls at 6h (appendix A–4.5, 1st chart). The following significant differences were found:

Table 4.6. Significant differences in slice weights

Assay	Lower assay	Higher assay	p	Cause
Slice w.	neg. controls, 24h (n=10)	neg. controls, 0h (n=10)	$p < 0.01$	Incubation time
Slice w.	neg. controls, 24h (n=10)	pos. controls, 24h (n=8)	$p < 0.05$	Menadione treatment

Slice weights decreased with longer treatment time. The main reasons for weight reduction of slices during dynamic organ culture incubation are wear and tear from the continuously stirred culture medium and the friction of slices against the metal mesh or other surface on which they rest. The outer cell layers are disrupted and torn off by the mechanical stress, and washed away by the medium. Cells disrupted by toxic influence and swelling can be washed away even more rapidly.

However, slice weights increased with menadione treatment (positive control). Many of the slices treated with menadione were visibly harder than the others after treatment. This may indicate an encapsulation of the outer cells as they reacted to the added toxin, and then a resistance to mechanical stress.

Menadione had a quite different effect than $FeSO_4$ on slice weights. This may reflect the two compounds' different modes of toxicity (Stubberfield and Cohen, 1989; Griffiths *et al.,* 1999). These differences may also influence on parameters that are normalised according to slice weights.

4.7.2. MTT results

Average MTT results ranged from 37.5% in positive controls at 24h, to 101.6% in $FeSO_4$ 400 µM at 24h (appendix A–4.5, 2nd chart). (Negative controls at 24h were set to 100%.) The following significant differences were found:

Table 4.7. Significant differences in MTT results

Assay	Lower assay	Higher assay	p	Cause
MTT	pos. controls, 24h (n=8)	neg. controls, 24h (n=10)	$p < 0.01$	Menadione treatment
MTT	$FeSO_4$ 5000 µM, 24h (n=4)	neg. controls, 24h (n=10)	$p < 0.01$	$FeSO_4$ treatment

Note: In the case of $FeSO_4$ 5000 µM at 24h versus negative controls at 24h, the difference was found using parametric analyses (ANOVA, t test). However, the MTT results in the $FeSO_4$ 5000 µM group failed the test of normality ($p < 0.05$), and non-parametric analyses (rank-ANOVA, rank sum test) showed no significant differences between the $FeSO_4$ 5000 µM group and other groups with respect to MTT ($p \geq 0.05$).

Large variations in the MTT results between experiments made it difficult to compare the results. To enable comparison of different experiments, the results were related to slice weights and then normalised against one of the control groups. Negative controls at 24 hours

was the only group included in the MTT test in all experiments, and so the results for this group was set to 100% in each experiment.

Slices treated with menadione (positive control) had a significantly lower ability to convert MTT to formazan. The viability of slices also seemed to decrease with high concentrations of $FeSO_4$ and with longer treatment times, but the results were not conclusive.

The MTT test appears to be more sensitive to menadione than to iron. Again this may reflect the two compounds' different modes of toxicity; oxidative stress and/or arylation of thiols by menadione (Stubberfield and Cohen, 1989), and free radical lipid peroxidation by iron (Griffiths *et al.,* 1999).

4.7.3. Enzyme leakage

4.7.3.1. ALAT results

Average ALAT leakage ranged from 4.7% in negative controls at 3h, to 20.2% in $FeSO_4$ 5000 µM at 24h (appendix A–4.5, 3rd chart). The ALAT leakage increased with longer treatment. However, no significant differences were found between any treatments ($p \geq 0.05$), possibly due to large standard errors (SE) resulting from different study designs and performance between experiments.

ALAT is a liver-specific enzyme in rats, but leakage of ALAT does not seem to be a very sensitive parameter for *in vitro* toxicity in rat liver slices based on these results.

4.7.3.2. ASAT results

Average ASAT leakage ranged from 1.8% in negative controls at 3h, to 14.4% in $FeSO_4$ 5000 µM at 24h (appendix A–4.5, 4th chart). These significant differences were found:

Table 4.8. Significant differences in ASAT leakage

Assay	Lower assay	Higher assay	p	Cause
ASAT leakage	neg. controls, 24h (n=10)	$FeSO_4$ 5000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment
ASAT leakage	neg. controls, 24h (n=10)	$FeSO_4$ 1000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment
ASAT leakage	$FeSO_4$ 100 µM, 24h (n=4)	$FeSO_4$ 5000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment

Assay	Lower assay	Higher assay	p	Cause
ASAT leakage	$FeSO_4$ 100 µM, 24h (n=4)	$FeSO_4$ 1000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment
ASAT leakage	$FeSO_4$ 100 µM, 3h (n=2)	$FeSO_4$ 100 µM, 24h (n=4)	$p < 0.05$	Incubation time
ASAT leakage	pos. controls, 3h (n=2)	pos. ctr., 6h (n=2), 24h (n=8)	$p < 0.01$	Incubation time
ASAT leakage	neg. controls, 3h (n=4)	neg. controls, 24h (n=10)	$p < 0.01$	Incubation time

ASAT leakage increased with time, but the results were almost the same in negative controls, positive controls, and $FeSO_4$ 100 µM.

With higher concentrations of $FeSO_4$ the ASAT leakage increased significantly. At 24 hours the ASAT leakage was much higher in slices treated with the highest concentrations of $FeSO_4$ (1000 and 5000 µM) compared to either negative controls or $FeSO_4$ 100 µM.

ASAT is less liver-specific than ALAT in rats, but the proportion of mitochondrial to cytosolic forms is greater with ASAT than with ALAT, and higher leakage of ASAT indicates more severe damage to subcellular organelles (Evans GO, 1996). Given that $FeSO_4$ promotes lipid peroxidation of organelles (Bacon *et al.*, 1986; Mak and Weglicki, 1985; Pushpendran *et al.*, 1998), ASAT leakage may be a sensitive marker of iron toxicity in tissue slices.

4.7.3.3. GLDH results

Average GLDH leakage ranged from 0.2% in negative controls at 3h, to 13.7% in $FeSO_4$ 5000 µM at 24h (appendix A–4.5, 5th chart). These significant differences were found:

Table 4.9. Significant differences in GLDH leakage

Assay	Lower assay	Higher assay	p	Cause
GLDH leakage	neg. controls, 24h (n=9)	$FeSO_4$ 5000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment
GLDH leakage	neg. controls, 24h (n=9)	$FeSO_4$ 1000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment
GLDH leakage	$FeSO_4$ 100 µM, 24h (n=4)	$FeSO_4$ 5000 µM, 24h (n=4)	$p < 0.05$	$FeSO_4$ treatment

Assay	Lower assay	Higher assay	p	Cause
GLDH leakage	$FeSO_4$ 100 µM, 3h (n=2)	$FeSO_4$ 100 µM, 24h (n=4)	$p < 0.05$	Incubation time
GLDH leakage	neg. controls, 3h (n=4)	neg. controls, 24h (n=9)	$p < 0.01$	Incubation time
GLDH leakage	pos. controls, 3h (n=2)	pos. controls, 24h (n=8)	$p < 0.01$	Incubation time

Note: In the experiments involving 200-1000-5000 µM $FeSO_4$, one sample mean was excluded from the negative controls at 24h (see explanation in section 4.6, page 57). Therefore n=9 instead of 10.

GLDH leakage increased significantly with longer treatment time, but the results were almost the same in negative controls, positive controls, and $FeSO_4$ 100 µM.

With higher concentrations of $FeSO_4$ the GLDH leakage was more pronounced, and the greatest difference in enzyme leakage between different iron concentrations is exhibited by GLDH. At 24 hours the leakage was 3.7 times higher with $FeSO_4$ 5000 µM than with 100 µM. The corresponding factors for the other enzymes at 24 hours are between 1.3 and 2.1. Based on this, GLDH leakage in liver slices may be particularly sensitive to iron toxicity.

Although the GLDH leakage was small, the relative increase in GLDH leakage from 3 hours to 24 hours was greater than for the other enzymes measured in the same period. In the groups observed at 3 and 24 hours – i.e. positive controls, negative controls, and $FeSO_4$ 100 µM – GLDH leakage grew by factors of 16, 18 and 19, respectively (see Table 4.10). Leakage of the other enzymes in the same period only grew by factors between 2.2 and 4.4. This indicates that GLDH may be a more sensitive marker than the other enzymes with respect to the length of treatment. GLDH is essentially a mitochondrial enzyme, and leakage of GLDH indicates severe organelle damage, which happens in the later stages of cellular damage (Evans GO, 1996).

Table 4.10. Increased enzyme leakage from 3 hours to 24 hours

The tabulated values correspond to the 3h and 24h bars in the enzyme leakage charts (appendix A–4.5; Pooled data from different study designs). The p values for the differences between 3h and 24h enzyme leakage are given elsewhere in section 4.7.3 (except from ALAT leakage for which p≥0.05).

	Leakage at 3h (%)			Leakage at 24h (%)			Factor 24h / 3h		
Enzyme	neg.c.	pos.c.	Fe-100	neg.c.	pos.c.	Fe-100	neg.c.	pos.c.	Fe-100
ALAT	4.7	6.7	4.6	11.3	14.7	11.1	2.4	2.2	2.4
ASAT	1.8	1.8	1.8	6.1	7.4	6.9	3.4	4.1	3.8
GLDH	0.16	0.16	0.20	2.9	2.5	3.7	18.	16.	19.
LDH	8.1	11.0	9.6	20.8	48.7	26.8	2.6	4.4	2.8

4.7.3.4. LDH results

Average LDH leakage ranged from 8.1% in negative controls at 3h, to 48.7% in positive controls at 24h (appendix A–4.5, 6th chart). The following significant differences were found:

Table 4.11. Significant differences in LDH leakage

Assay	Lower assay	Higher assay	p	Cause
LDH leakage	neg. controls, 24h (n=10)	pos. controls, 24h (n=8)	$p < 0.01$	Menadione treatment
LDH leakage	neg. controls, 6h (n=4)	pos. controls, 6h (n=2)	$p < 0.01$	Menadione treatment
LDH leakage	$FeSO_4$ 100 µM, 3h (n=2)	$FeSO_4$ 100 µM, 24h (n=4)	$p < 0.05$	Incubation time
LDH leakage	pos. controls, 3h (n=2)	pos. controls, 24h (n=8)	$p < 0.01$	Incubation time
LDH leakage	neg. controls, 3h (n=4)	neg. controls, 24h (n=10)	$p < 0.01$	Incubation time

LDH leakage was almost the same in $FeSO_4$ 100 µM and negative controls at all time points, and higher concentrations of $FeSO_4$ did not make a big difference.

On the other hand, LDH leakage increased markedly with menadione treatment.
The differences in LDH leakage between menadione and negative controls, and also between menadione for 24h and 3h, suggest that LDH leakage is very sensitive to menadione treatment. This was also shown in individual experiments involving menadione (results in sections 4.3 p. 53, and 4.5 p. 56).

The results of the present study agree with Leeman *et al.* (1995) who found that LDH leakage was significantly higher in slices treated for 4 hours with 100, 200 or 400 µM menadione than in negative controls. LDH has low organ specificity and is mainly cytosolic, and LDH leakage happens early in cellular damage (Evans GO, 1996). LDH leakage seems to be more sensitive to menadione than to $FeSO_4$.

4.7.3.5. Summary of enzyme leakage results

Disruption of cells due to mechanical or chemical factors leads to enzyme leakage as well as weight reduction. In early stages of tissue damage, cytoplasmic enzymes leak from cells when plasma membrane permeability is altered. As the tissue damage becomes more severe, enzymes normally present in subcellular organelles will also be released (Evans GO, 1996). This is reflected by the cytosolic enzymes ALAT and LDH leaking 5-10% already at 3 hours and up to 20% and 50% at 24 hours, respectively, whereas the mitochondrial enzymes GLDH and ASAT only leak 0.2-1.8% at 3 hours and less than 15% at 24 hours in the present study.

Leakage of all enzymes increased with longer treatment time and with chemical treatment, but for ALAT leakage the differences were not significant. ASAT and GLDH leakage were most sensitive to iron treatment, while LDH leakage was most sensitive to menadione treatment.

4.7.4. Potassium content

Average K content ranged from 77.0% in positive controls at 24h, to 269% in negative controls at 6h (appendix A–4.5, 7th chart). (Negative controls at 0h were set to 100%.) The following significant differences were found:

Table 4.12. Significant differences in K content

Assay	Lower assay	Higher assay	p	Cause
K	neg. controls, 0h (n=8)	neg. controls, 3h (n=2), 6h (n=2), 24h (n=8)	$p < 0.01$	Medium and incubation time
K	pos. controls, 24h (n=6)	neg. controls, 24h (n=8)	$p < 0.01$	Menadione treatment
K	$FeSO_4$ 1000 µM, 24h (n=4)	neg. controls, 24h (n=8)	$p < 0.01$	$FeSO_4$ treatment
K	$FeSO_4$ 5000 µM, 24h (n=4)	neg. controls, 24h (n=8)	$p < 0.01$	$FeSO_4$ treatment

The K content in slices increased significantly from 0 hours to 3 hours in negative controls. Increased K content during the first hours of incubation may be caused by potassium in the culture medium (the medium contained the buffer $NaHPO_4$ / KH_2PO_4 plus KCl) or by a transitory potassium leakage during slice preparation and subsequent recovery. If the latter is the case, the recovery must take longer time than the one hour of pre-incubation but is probably complete within 3 to 6 hours, according to the peak in K content at 6 hours.

The K content decreased with chemical treatment, or did not increase as much as in negative controls. Compared to negative controls, K content was significantly lower with 1000 µM and 5000 µM $FeSO_4$, and even lower with menadione (positive controls). This may be caused by damage to the plasma membrane or a block in ATP synthesis impairing energy-dependent cellular ion pumping mechanisms, allowing sodium and calcium to enter and potassium to escape (Vickers, 1997).

4.7.5. Iron content

Iron content was not a marker of toxicity in itself but may contribute in the evaluation of the other assays.

Average Fe content ranged from 63.3% in positive controls at 24h, to 218% in $FeSO_4$ 5000 µM at 24h (appendix A–4.5, last chart). (Negative controls at 0h were set to 100%.) The following significant differences were found:

Table 4.13. Significant differences in Fe content

Assay	Lower assay	Higher assay	p	Cause
Fe	neg. controls, 24h (n=8)	$FeSO_4$ 5000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment
Fe	$FeSO_4$ 100 µM, 24h (n=4)	$FeSO_4$ 5000 µM, 24h (n=4)	$p < 0.01$	$FeSO_4$ treatment

Note: These differences were found using parametric analyses (ANOVA, t test). However, all treatment groups failed the test on homogeneity of variance ($p < 0.05$), and the additional non-parametric analyses (rank-ANOVA, rank sum test) showed no significant differences in Fe content between any groups ($p \geq 0.05$).

Iron content was reasonably constant in all groups except from $FeSO_4$ 5000 µM at 24 hours. Although not significant due to non-homogeneity of variance, the iron content in slices from the $FeSO_4$ 5000 µM group at 24 hours is higher than in $FeSO_4$ 100 µM and in negative control at the same time.

The increased Fe content is expected, but it may have several explanations; significant uptake of soluble iron into cells, adsorption of iron onto the surface of slices without cellular uptake, or excess iron in culture medium not rinsed away properly before assaying. The toxic effects observed with high concentrations of iron in medium (i.e. MTT test, ASAT leakage, GLDH leakage, and K leakage), in particular the leakage of mitochondrial enzymes, suggest that the iron was indeed absorbed and exerted its effects inside the cells.

5. CONCLUSION

A new incubator at GE Healthcare was tested with rat liver slices and various concentrations of menadione and iron (II) sulphate in William's medium E. The operation of the equipment was relatively straightforward, albeit time consuming. And the quality of slices did not seem to have improved since previous studies at GE Healthcare, illustrated by large variation in results between different experiments and also the poor quality of histology samples. More experiments and more practical training of personnel in using the equipment is required to improve the performance of the rat liver slice model.

To obtain toxic effects on tissue slices *in vitro*, where most physiological processes are not present in the test model, the test substances must be directly cytotoxic. Isolated hepatocytes may be easier to culture and often used for toxicity screening, and liver slices may be more useful for studies on metabolism and for inter-species comparison.

Certain *in vitro* assays for toxicity in rat liver slices, particularly enzyme leakage and potassium (K) content, were found to be sensitive to treatment with iron (II) sulphate and menadione. Some effects observed in the rat liver slices treated with these substances were:

- LDH leakage increased significantly with exposure to menadione
- ASAT leakage and GLDH leakage increased significantly with iron (II) sulphate
- K content decreased significantly with iron (II) sulphate or menadione treatment

Of the assays evaluated in the present study, GLDH leakage seemed to be the most predictive of iron (II) sulphate toxicity in rat liver slice *in vitro*, and LDH leakage the most predictive of menadione toxicity.

REFERENCES

Bach PH, Vickers AEM, Fisher R, *et al.* (1996): *The use of tissue slices for pharmaco-toxicology studies. The report and recommendations of ECVAM* workshop 20.* Alternatives to Laboratory Animals 24:893-923.

Bacon BR, O'Neill R, and Park CH (1986): *Iron-induced peroxidative injury to isolated rat hepatic mitochondria.* Journal of Free Radical Biology and Medicine 2:339-347.

Berridge MV, and Tan AS (1993): *Characterisation of the cellular reduction of 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular localisation, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction.* Archives of Biochemistry and Biophysiology 303:474-482.

Berry MN, Edwards AM, and Barritt GJ (1991): *Isolated hepatocytes: Preparation, properties and applications.* Elsevier, New York.

Borenfreund E, Babich H, and Martin-Alguacil N (1988): *Comparison of two in vitro cytotoxicity assays – the neutral red (NR) and tetrazolium MTT tests.* Toxicology in Vitro 2:1-6.

Burge JA, and Aust SD (1978): *Microsomal lipid peroxidation.* Methods in Enzymology 52:302-310.

Chan HM, Tabarrok R, Tamura Y, and Cherian MG (1992): *The relative importance of glutathione and metallothionein on protection of hepatotoxicity of menadione in rats.* Chemico-Biological Interactions 84:113-124.

DeLuca M, and McElroy ND (1978): *Purification and properties of firefly luciferase.* Methods in Enzymology 57:3-14.

Deutsche Gesellschaft für Klinische Chemie (1972): *Empfehlungen der Deutschen Gesellschaft für Klinische Chemie.* Journal of Clinical Chemistry and Clinical Biochemistry 10:182-193.

Dogterom P (1993): *Development of a simple incubation system for metabolism studies with precision-cut liver slices.* Drug Metabolism and Disposition 21:699-704.

Downs TR, and Wilfinger WW (1983): *Fluorometric quantification of DNA in cells and tissue.* Analytical Biochemistry 131:538-547.

Evans GO (1986): *General enzymology.* In: Animal Clinical Chemistry – A Primer for Toxicologists, edited by Evans GO, pp. 59-67, Taylor & Francis Ltd, London.

Fisher RL, Shaugnessy RP, Jenkins PM, Austin ML, Roth GL, Gandolfi AJ, and Brendel K (1995a): *Dynamic organ culture is superior to multiwell plate culture for maintaining precision-cut tissues: Optimisation of tissue slice culture, part 1.* Toxicological Methods 5:99-113.

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* ECVAM: European Centre for the Validation of Alternative Methods, JRC Environment Inst., Ispra, Italy

Fisher RL, Hasal SJ, Sanuik JT, Gandolfi AJ, and Brendel K (1995b): *Determination of optimal incubation media and suitable slice diameters in precision-cut liver slices: Optimisation of tissue slice culture, part 2.* Toxicological Methods 5:115-130.

Gandolfi AJ, Brendel K, Fisher RL, and Michaud JP (1995): *Use of tissue slices in chemical mixture toxicology and interspecies investigations.* Toxicology 105:285-290.

Griffiths WJ, Kelly AL, and Cox TM (1999): *Inherited disorders of iron storage and transport.* Molecular Medicine Today 5:431-438.

Grisham JW (1979): *Use of hepatic cell cultures to detect and evaluate the mechanisms of action of toxic chemicals.* International Reviews on Experimental Pathology 20:123-210.

Hissin PJ, and Hilf R (1976): *A fluorometric method for determination of oxidised and reduced glutathione in tissues.* Analytical Biochemistry 74:214-226.

Hodgson E (2001): *Hepatotoxicology. Introduction.* In: Current Protocols in Toxicology, edited by Maines MD *et al.,* p. 14.0.1, John Wiley & Sons, New York.

International Federation of Clinical Chemistry (1986 a, b): Journal of Clinical Chemistry and Clinical Biochemistry 24:481-495, *ibid.* 24:497-510.

Jones DP (1981): *Determination of pyridine dinucleotides in cell extracts by high-performance liquid chromatography.* Journal of Chromatography 225:446-449.

de Kanter R, and Koster HJ (1995): *Cryopreservation of rat and monkey liver slices.* Alternatives to Laboratory Animals 23:653-665.

Kimmick GA, Randles J, and Brand JS (1975): *Assay of picomole amounts of ATP, ADP, and AMP using the luciferase enzyme system.* Analytical Biochemistry 69:187-206.

Krumdieck CL, dos Santos JE, and Ho KJ (1980): *A new instrument for the rapid preparation of tissue slices.* Analytical Biochemistry 104:118-123.

Leeman WR, van de Gevel IA, and Rutten AAJJL (1995): *Cytotoxicity of retinoic acid, menadione and aflatoxin B_1 in rat liver slices using netwell inserts as a new culture system.* Toxicology in Vitro 9:291-298.

Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ (1951): *Protein measurement with the folin phenol reagent.* Journal of Biological Chemistry 193:265-275.

Lundin A, Hasenson M, Persson J, and Pousette A (1986): *Estimation of biomass in growing cell lines by adenosine triphosphate assay.* Methods in Enzymology 133:27-42.

Lundin A (2000): *Use of firefly luciferase in ATP-related assays of biomass, enzymes, and metabolites.* Methods in Enzymology 305:346-370.

Mak IT, and Weglicki WB (1985): *Characterisation of iron-mediated peroxidative injury in isolated hepatic lysosomes.* Journal of Clinical Investigation 75:58-63.

McGuinness S, Gandolfi AJ, and Brendel K (1993): *Use of renal slices and renal tubule suspensions for in vitro toxicity studies.* In Vitro Toxicology 6:1-24.

McQueen CA (1993): *Isolation and culture of hepatocytes from different laboratory species.* In: In Vitro Biological Systems, edited by Tyson CA and Frasier GN, pp. 255-270, Academic Press, New York.

Mehendale HM (1991): *Commentary. Role of hepatocellular regeneration and hepatolobular healing in the final outcome of liver injury: A two-stage model of toxicity.* Biochemical Pharmacology 42:1155-1161.

Merck Index (1996), 12th edition, edited by Budavari S *et al.,* Merck & Co., Whitehouse Station, New Jersey.

Meyer SA (2001): *Overview of hepatotoxicity.* In: Current Protocols in Toxicology, edited by Maines MD *et al.,* pp. 14.1.1-14.1.6, John Wiley & Sons, New York.

Miller MG, Beyer J, Hall GL, de Graffenried LA, and Adams PE (1993): *Predictive value of liver slices for metabolism and toxicity in vivo: Use of acetaminophen as a model hepatotoxicant.* Toxicology and Applied Pharmacology 122:108-116.

Mosmann T (1983): *Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays.* Journal of Immunological Methods 65:55-63.

Mudra DR, and Parkinson A (2001): *Preparation of hepatocytes.* In: Current Protocols in Toxicology, edited by Maines MD *et al.,* pp. 14.2.1-14.2.13, John Wiley & Sons, New York.

Obatomi DK, Brant S, Anthonypillai V, and Bach PH (1998a): *Toxicity of atractyloside in precision-cut rat and porcine renal and hepatic tissue slices.* Toxicology and Applied Pharmacology 148:35-45.

Obatomi DK, Thanh NTK, Brant S, and Bach PH (1998b): *The toxic mechanism and metabolic effects of atractyloside in precision-cut pig kidney and liver slices.* Archives of Toxicology 72:524-530.

Obatomi DK, Brant S, Anthonypillai V, Early DA, and Bach PH (1998c): *Optimising pre-incubation conditions for precision-cut rat kidney and liver tissue slices: Effect of culture media and antioxidants.* Toxicology in Vitro 12:725-737.

Olinga P, Groen K, Hof IH, de Kanter R, Koster HJ, Leeman WR, Rutten AAJJL, van Twillert K, and Groothuis GMM (1997): *Comparison of five incubation systems for rat liver slices using functional and viability parameters.* Journal of Pharmacological and Toxicological Methods 38:59-69.

Parrish AR, Gandolfi AJ, and Brendel K (1995): *Minireview. Precision-cut tissue slices: Applications in pharmacology and toxicology.* Life Sciences 57:1887-1901.

Plaa GL, and Charbonneau M (1994): *Detection and evaluation of chemically induced liver injury.* In: Principles and Methods of Toxicology, 3rd edition, edited by Hayes AW, pp. 839-870, Raven Press Ltd., New York.

Price RJ, Mistry H, Wield PT, Renwick AB, Beamand JA, and Lake BG (1996): *Comparison of the toxicity of allyl alcohol, coumarin and menadione in precision-cut rat, guinea-pig, Cynomolgus monkey and human liver slices.* Archives of Toxicology 71:107-111.

Price RJ, Ball SE, Renwick AB, Barton PT, Beamand JA, and Lake BG (1998): *Use of precision-cut rat liver slices for studies of xenobiotic metabolism and toxicity: Comparison of the Krumdieck and Brendel tissue slicers.* Xenobiotica 28:361-371.

Pushpendran CK, Subramanian M, Devasagayam TP, and Singh BB (1998): *Study on lipid peroxidation potential in different tissues induced by ascorbate-Fe^{2+}: Possible factors involved in their differential susceptibility.* Molecular and Cellular Biology 178:197-202.

Ruegg CE, Gandolfi AJ, Nagle RB, Krumdieck CL, and Brendel K (1987): *Preparation of positional renal slices for study of cell-specific toxicity.* Journal of Pharmacological Methods 17:111-123.

Ruegg CE (1994): *Preparation of precision-cut renal slices and renal proximal tubular fragments for evaluating segment-specific toxicity.* Journal of Pharmacological and Toxicological Methods 31:125-133.

Sipes IG, Fisher RL, Smith PF, Stine ER, Gandolfi AJ, and Brendel K (1987): *A dynamic liver culture system: A tool for studying chemical biotransformation and toxicity.* Archives of Toxicology 11(supplement):20-33.

Smith PF, Gandolfi AJ, Krumdieck CJ, Putnam CW, Zukoski CF, Davis WM, and Brendel K (1985): *Dynamic organ culture of precision liver slices for in vitro toxicology.* Life Sciences 36:1367-1375.

Smith PF, Krack G, McKee RL, Johnson DG, Gandolfi AJ, Hruby VJ, Krumdieck CL, and Brendel K (1986): *Maintenance of adult rat liver slices in dynamic organ culture.* In Vitro Cellular and Developmental Biology 22:706-712.

Société Française de Biologie Clinique (1982 a, b, c): Annales de Biologie Clinique 40:154-159, *ibid.* 40:132-138, *ibid.* 40:160-164.

Stubberfield CR, and Cohen GM (1989): *Interconversion of NAD(H) to NADP(H): A cellular response to quinone-induced oxidative stress in isolated hepatocytes.* Biochemistry and Pharmacology 38:2631-2637.

Utley WS, and Mehendale HM (1989): *Phenobarbital-induced cytosolic cytoprotective mechanisms that offset increases in NADPH cytochrome P450 reductase activity in menadione-mediated cytotoxicity.* Toxicology and Applied Pharmacology 99:323-333.

Vickers AEM (1997): *Experimental in vitro models to evaluate hepatotoxicity.* In: In Vitro Methods in Pharmaceutical Research, edited by Castell JV and Gómez-Lechón MJ, pp. 103-127, Academic Press Ltd., New York.

Williams GM, Weisburger EK, and Weisburger JH (1971): *Isolation and long-term cell culture of epithelial-like cells from rat liver.* Experimental Cell Research 69:106-112.

Wright MC, and Paine AJ (1992): *Resistance of precision-cut liver slices to the toxic effects of menadione.* Toxicology in Vitro 6:475-481.

Wright TL, Brissot P, Ma WL, and Weisiger RA (1986): *Characterisation of non-transferrin-bound iron clearance by rat liver.* Journal of Biological Chemistry 261:10909-10914.

Wright TL, and Lake JR (1990): *Mechanisms of transport of non-transferrin-bound iron in basolateral and canalicular rat liver plasma membrane vesicles.* Hepatology 12:498-504.

APPENDIX

A–1. MANUFACTURERS AND SUPPLIERS OF MATERIALS

Short name	Full name and address of headquarters or affiliate
AGA	AGA, Strømsveien 324, PB 123 Leirdal, N-1081 Oslo, Norway
Alabama	Alabama Research & Development, P.O.Box 739, Munford, Alabama 36268 USA
Arcus	Arcus Produkter, Haslevangen 16, PB 6764 Rodeløkka, N-0503 Oslo, Norway
Baxter	Baxter Healthcare, 95 Spring Street, New Providence, New Jersey 07974 USA
B&K	B & K Universal, Box 6023, S-19106 Sollentuna, Sweden
BioWhit	BioWhittaker, 8830 Biggs Ford Road, P.O.Box 127, Walkersville, MD 21793 USA
Braun	B. Braun Medical, CH-6020 Emmenbrücke, Switzerland
ChemScan	ChemScan, Grindalsveien 14, N-2400 Elverum, Norway
Desaga	Desaga Sarstedt Gruppe, D-69123 Heidelberg, Germany
Dynatech	Dynatech Laboratories, 14340 Sullyfield Circle, Chantilly, Virginia 22021 USA
Hamilton	Hamilton Bonaduz, CH-7402 Bonaduz, Switzerland
Harvard	Harvard Apparatus, 84 October Hill Road, Holliston, Massachusetts 01746 USA
Histolab	Histolab Products, Hulda Lindgrensgata 6, S-42131 Västra Frölunda, Sweden
Leica	Leica Microsystems, Imneuenheimer Feld 518, D-69120 Heidelberg, Germany
LifeTech	Life Technologies, 9800 Medical Center Dr, POB 6482, Rockville, MD 20849 USA
Merck	E. Merck, Frankfurterstrasse 250, D-64293 Darmstadt, Germany
Mettler	Mettler Instruments, 1900 Polaris Parkway, Columbus, Ohio 43240 USA
Nat.Diag	National Diagnostics, 305 Patton Drive, Atlanta, Georgia 30336 USA
NIST	National Institute of Standards & Technology, 100 Bureau Drive, Stop 3460, Gaithersburg, Maryland 20899 USA
Nunc	Nunc, Postbox 280 Kamstrup, DK-4000 Roskilde, Denmark
Packard	Packard Instrument, 800 Research Parkway, Meriden, Connecticut 06450 USA
PerkinE	PerkinElmer Instruments, 710 Bridgeport Avenue, Shelton, CT 06484 USA
Roche	Roche Diagnostics, D-68298 Mannheim, Germany
Sakura	Sakura / Bayer Diagnostics, Bayer Norway, PB 311, N-1324 Lysaker, Norway
Sarstedt	Sarstedt, D-51588 Nümbrecht, Germany
Schleicher	Schleicher & Schuell, Postfach 4, D-37582 Dassel, Germany
Sigma	Sigma-Aldrich Norway, Tevlingveien 23, PB 188 Leirdal, N-1011 Oslo, Norway
Sigma-D	Sigma Diagnostics, St. Louis, Missouri 63178 USA
Skil	Skil Europe, Konijnenberg 60, Breda, Netherlands
SDS	Special Diets Services, Postbox 180, NL-5830 AD Boxmeer, Netherlands
Teknolab	Teknolab, PB 131, N-1441 Drøbak, Norway
Vitron	Vitron, 8320 South Wentworth Road, Tucson, Arizona 85747 USA
Vogel	Vogel medizinischer Technik und Elektronik, Marburgerstr. 81, D-35396 Glessen

A–2. SUMMARY OF ANIMAL CHARACTERISTICS

Summary of animal characteristics

All animals were male Sprague-Dawley rats (type BKL from B&K), except animal 0389-01 which was female.
The animals were starved for the last part of the last night, except animals 13xx-00 which were fed normally.
"Arrival weight" was measured the morning after arrival, except for animal 063x-01 which was measured two days after arrival.
One animal was used in each experiment, except in EGN-05-01 where two animals were used.
Highlighted experiments are presented in the thesis. The other experiments were excluded as explained in the results chapter.

Study #	Animal #	Order weight (g)	Order age (week)	Birth date	Arrival date	Arrival age (week)	Arrival weight (g)	Study date	Study age (week)	Body weight (g)	Acclimatisation (days, weightgain)		Liver weight (g)	Liver % of body
EGN-01-00	1335-00	200		17-sep-00	25-okt-00	5,43	172	31-okt-00	6,29	218	6	27 %	9,6	4,4 %
EGN-02-00	1371-00	200		21-sep-00	8-nov-00	6,86	152	14-nov-00	7,71	200	6	32 %	11,1	5,6 %
EGN-03-00	1385-00	200		10-okt-00	22-nov-00	6,14	198	27-nov-00	6,86	245	5	24 %	10,2	4,2 %
EGN-04-00	**1444-00**	**200**		**26-okt-00**	**6-des-00**	**5,86**	**196**	**11-des-00**	**6,57**	**215**	**5**	**10 %**	**7,4**	**3,4 %**
EGN-01-01	0204-01	150		28-nov-00	3-jan-01	5,14	154	10-jan-01	6,14	192	7	25 %	6,1	3,2 %
EGN-02-01	**0210-01**	**210**		**11-des-00**	**24-jan-01**	**6,29**	**199**	**31-jan-01**	**7,29**	**249**	**7**	**25 %**	**9,3**	**3,7 %**
EGN-03-01	**0321-01**	**210**	**6**	**29-des-00**	**7-feb-01**	**5,71**	**195**	**14-feb-01**	**6,71**	**238**	**7**	**22 %**	**8,4**	**3,5 %**
EGN-04-01	0389-01	210	6	31-des-00	21-feb-01	7,43	204	27-feb-01	8,29	203	6	0 %	6,0	3,0 %
EGN-05-01	**0473-01**	**210**	**6**	**24-jan-01**	**7-mar-01**	**6,00**	**212**	**14-mar-01**	**7,00**	**240**	**7**	**13 %**	**8,1**	**3,4 %**
EGN-05-01	**0474-01**	**210**	**6**	**24-jan-01**	**7-mar-01**	**6,00**	**217**	**14-mar-01**	**7,00**	**245**	**7**	**13 %**	**8,5**	**3,5 %**
EGN-06-01	**0472-01**	**150-180**		**28-jan-01**	**7-mar-01**	**5,43**	**170**	**21-mar-01**	**7,43**	**257**	**14**	**51 %**	**7,8**	**3,0 %**
EGN-07-01	**0478-01**	**210**	**6**	**11-feb-01**	**21-mar-01**	**5,43**	**206**	**29-mar-01**	**6,57**	**258**	**8**	**25 %**	**9,8**	**3,8 %**
EGN-08-01	0483-01	210	6	16-feb-01	28-mar-01	5,71	209	4-apr-01	6,71	254	7	22 %	9,5	3,7 %
EGN-09-01	**0584-01**	**160**	**6**	**19-mar-01**	**25-apr-01**	**5,29**	**163**	**7-mai-01**	**7,00**	**260**	**12**	**60 %**	**9,5**	**3,7 %**
EGN-10-01	**0617-01**	**210**	**6**	**1-apr-01**	**9-mai-01**	**5,43**	**216**	**14-mai-01**	**6,14**	**246**	**5**	**14 %**	**9,3**	**3,8 %**
EGN-11-01	**0636-01**	**210-230**	**6**	**15-apr-01**	**23-mai-01**	**5,43**	**233**	**28-mai-01**	**6,14**	**240**	**5**	**3 %**	**8,0**	**3,3 %**
EGN-12-01	**0637-01**	**210-230**	**6**	**15-apr-01**	**23-mai-01**	**5,43**	**226**	**30-mai-01**	**6,43**	**255**	**7**	**13 %**	**8,9**	**3,5 %**

A–3. THE HEMATOXYLIN / EOSIN STAINING PROCESS

This staining process was used in section 3.3.6 Histology, page 47. An automatic slide stainer (DRS-60 from Sakura) carried out the process in accordance with the following scheme set up at GE Healthcare, Dept. of Regulatory Toxicology. Dyes and ammonia were stored in tight containers between runs. Dyes were filtered before every run and changed every 20 runs. Other solutions were changed depending on use and degree of discoloration (e.g., solutions no. 11, 14, and 15 picked up colour quickly).

Step	Time		Solution (supplier, article no.)	Notes
	min	sec		
1	5		Histoclear (HS-202 from Nat.Diag)	Dissolve remaining wax
2	5		Histoclear	
3	1		100% ethanol (1004857 from Arcus)	Prepare hydration
4	1		100% ethanol	
5	1		96% ethanol (1003203 from Arcus)	
6	1		96% ethanol	
7	1		80% ethanol*	
8	1		Running water	
9	8		Harris hematoxylin (01800, Histolab)†	Basic blue dye staining nuclei
10	10		Running water	Wash off excess dye
11		10	1% ammonia solution‡	Alkalinise to fixate dyes
12	10		Running water	Wash off excess ammonia
13	6		Eosin Y (HT110-1-32 from Sigma-D)§	Acid red dye staining cytoplasm
14		20	96% ethanol	Wash off excess dye; dehydrate
15		20	96% ethanol	
16		20	100% ethanol	
17		20	100% ethanol	
18		20	Histoclear	
19	1		Histoclear	
20	1		Histoclear	
21	1		Histoclear	Dehydration complete
sum	**54**	**50**	**plus transfer time between steps**	

* 80% ethanol: Prepare using 100% ethanol (1004857 from Arcus) and RO-water.

† This is a water- and propylene glycol-based solution of 0.4% hematoxylin and additives.

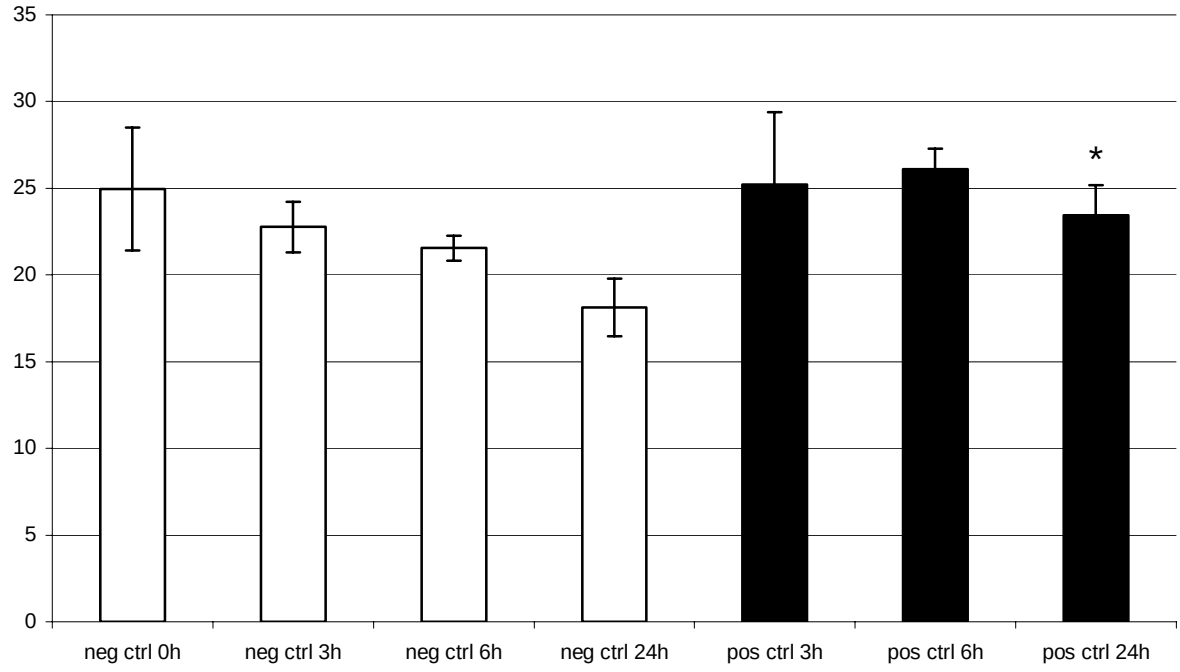
‡ 1% ammonia solution: Prepare using 25% ammonia solution (1.05432 from Merck) and RO-water.

§ This is an ethanol-based solution of eosin Y, or eosin yellowish–(YS).

A–4. GRAPHS FOR THE RESULTS AND DISCUSSION

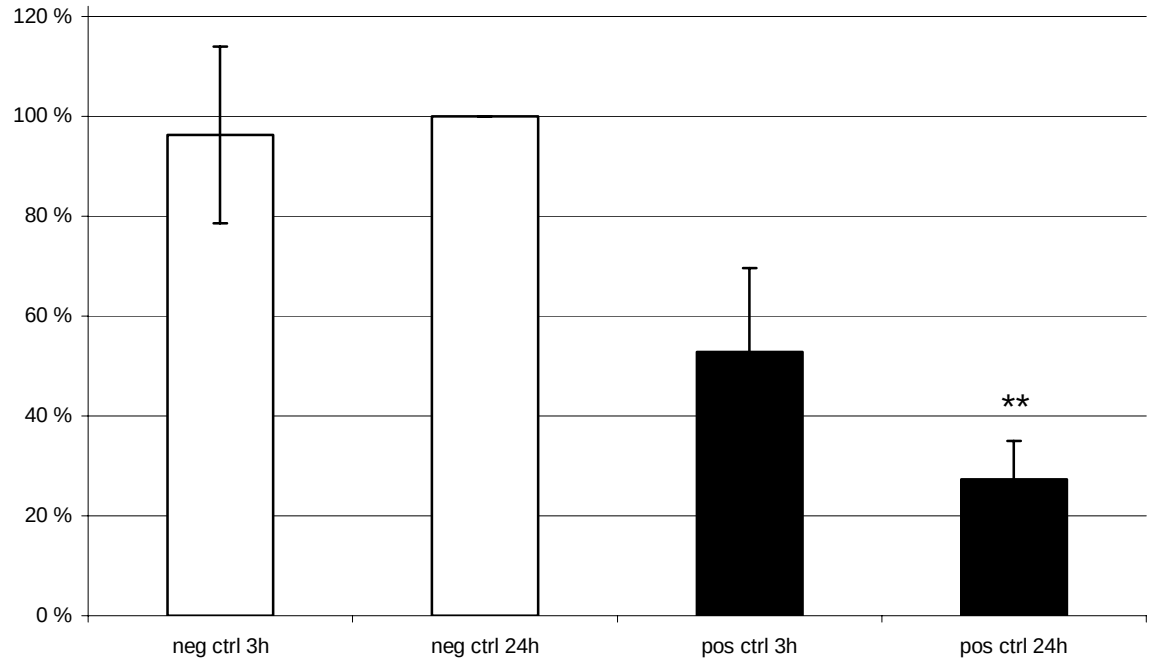
A–4.1. Menadione 200 µM (see legend p. 52, discussion p. 53)

Slice weights (mg)



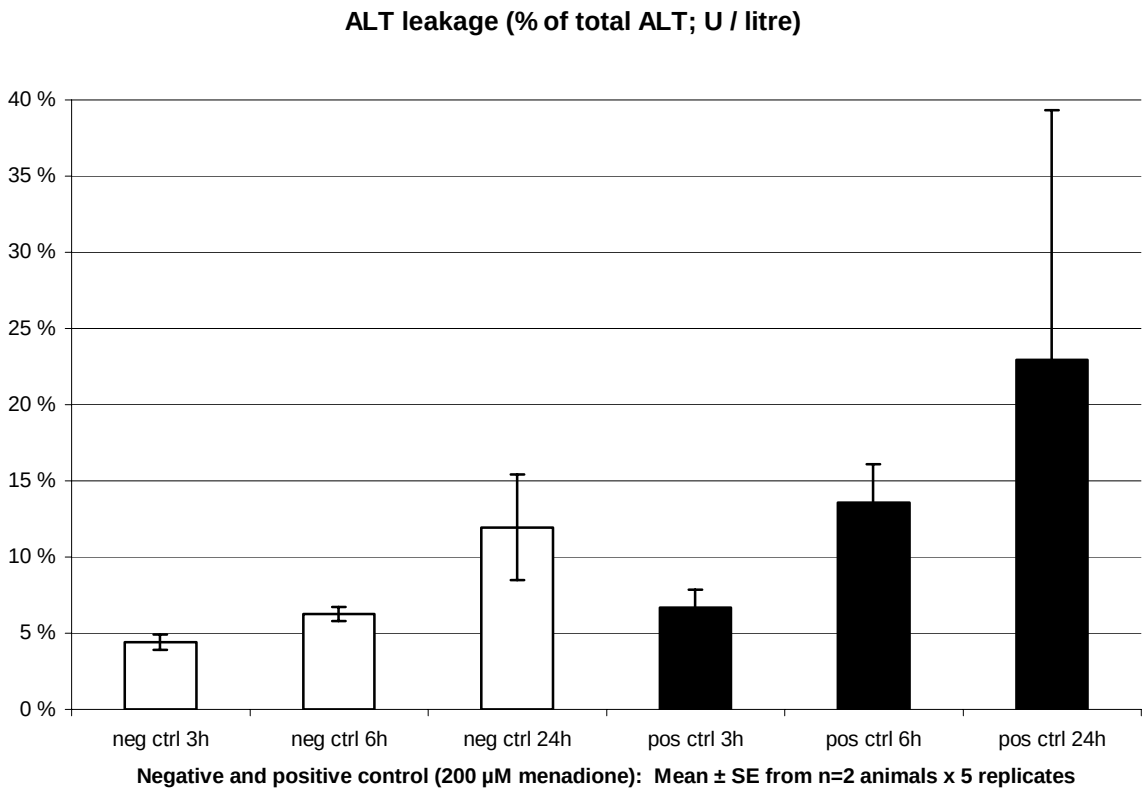
Negative and positive control (200 µM menadione): Mean ± SE from n=2 animals x 5 replicates

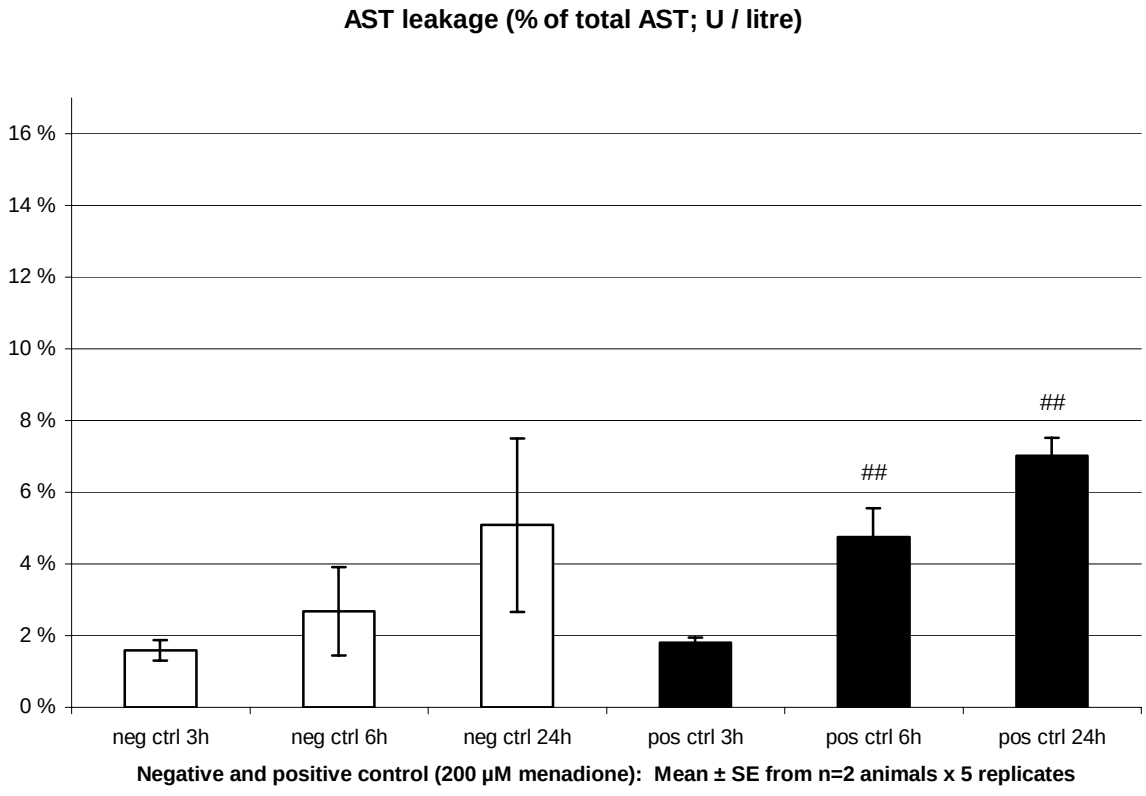
MTT test (% of 24h negative control; OD-570 / mg slice weight)



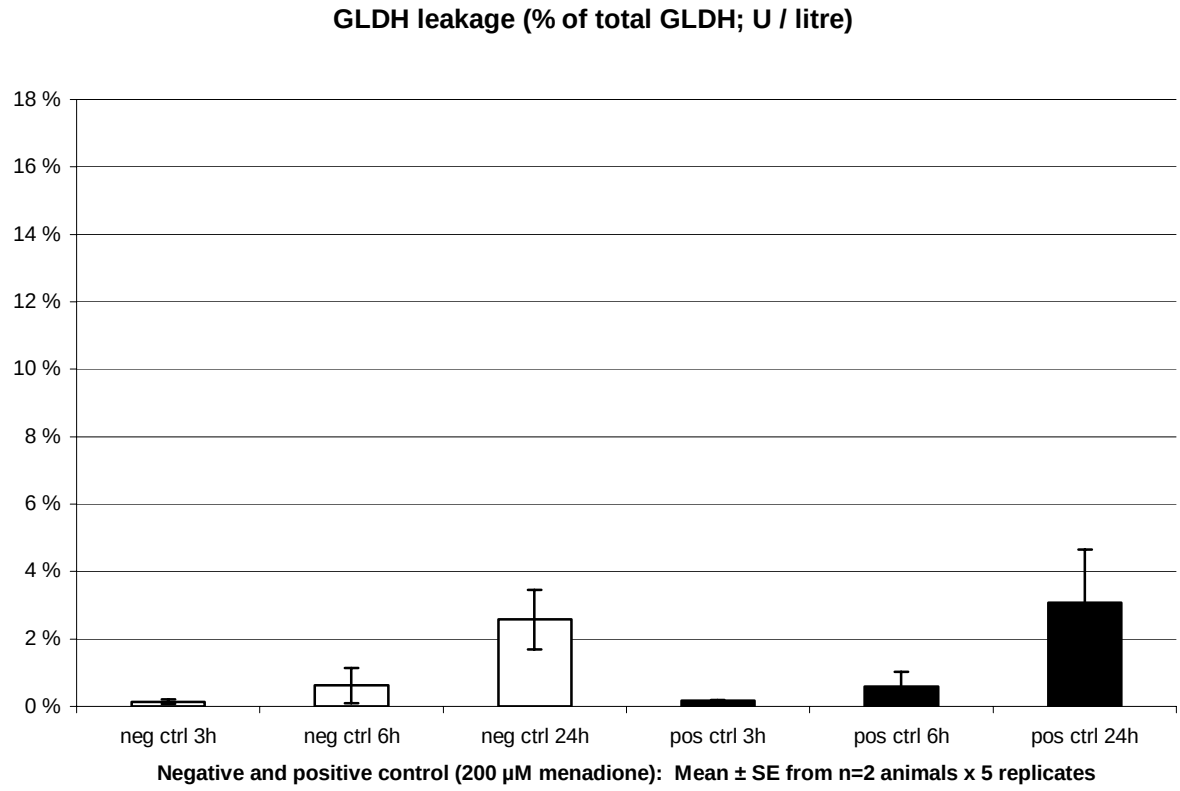
Negative and positive control (200 µM menadione): Mean ± SE from n=2 animals x 2 replicates

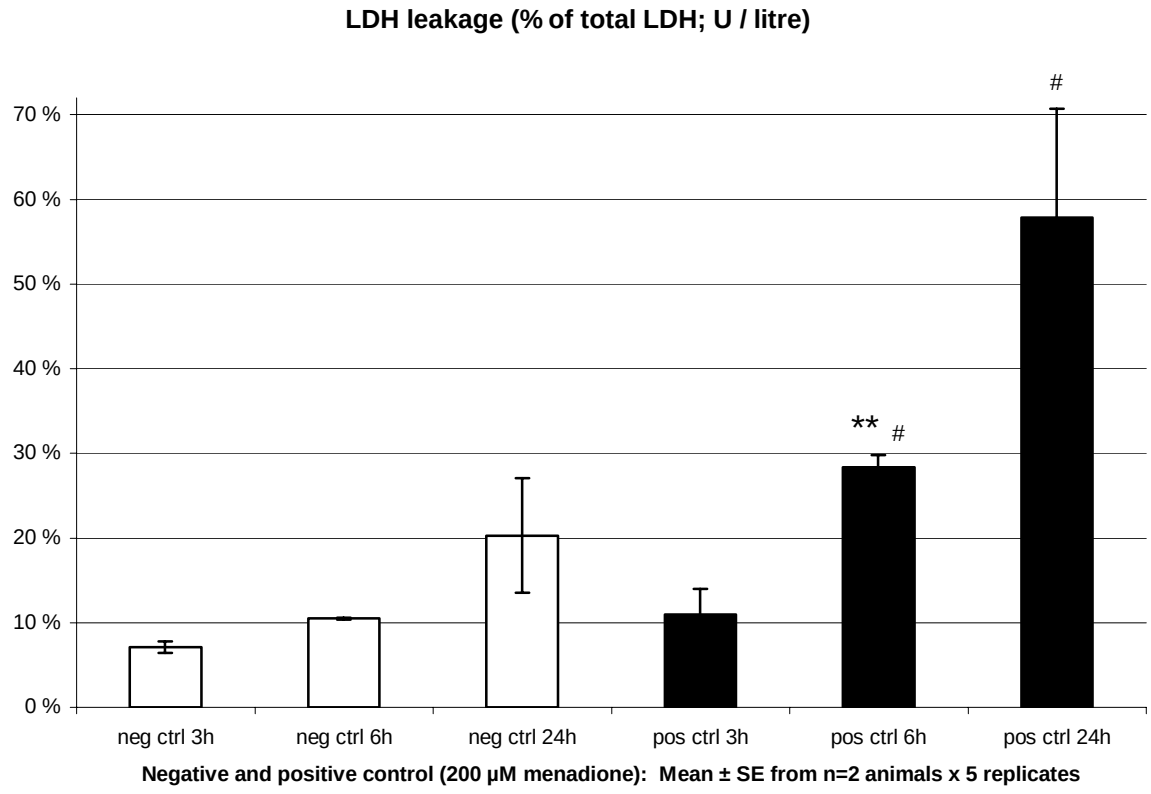
Menadione 200 µM (continued; see legend p. 52, discussion p. 53)





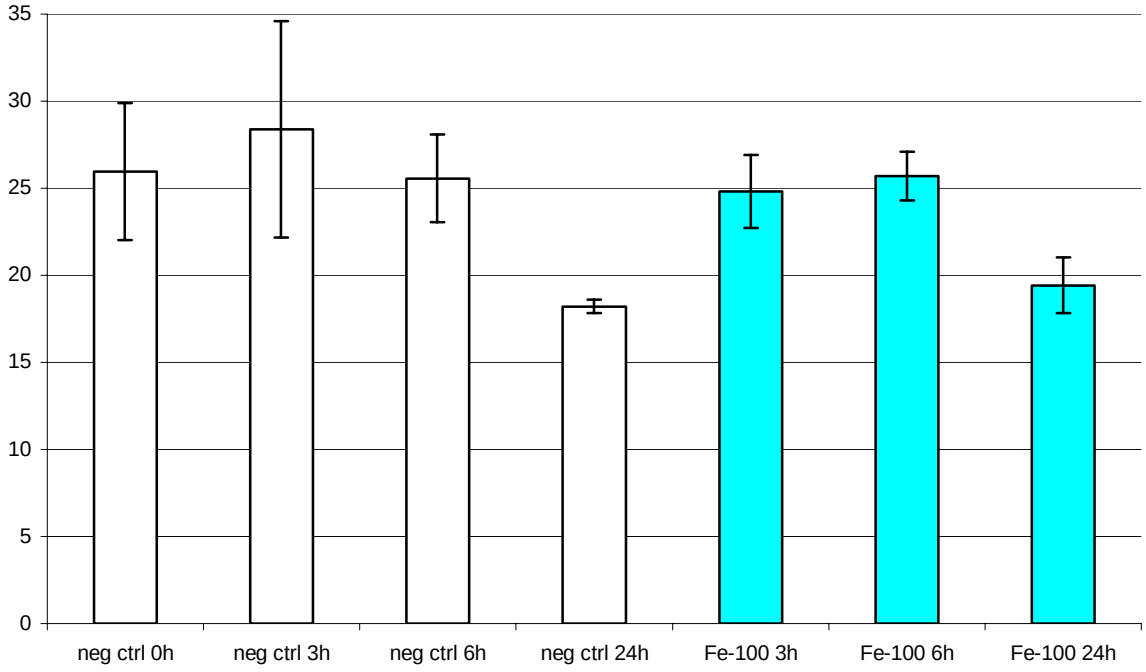
Menadione 200 µM (continued; see legend p. 52, discussion p. 53)





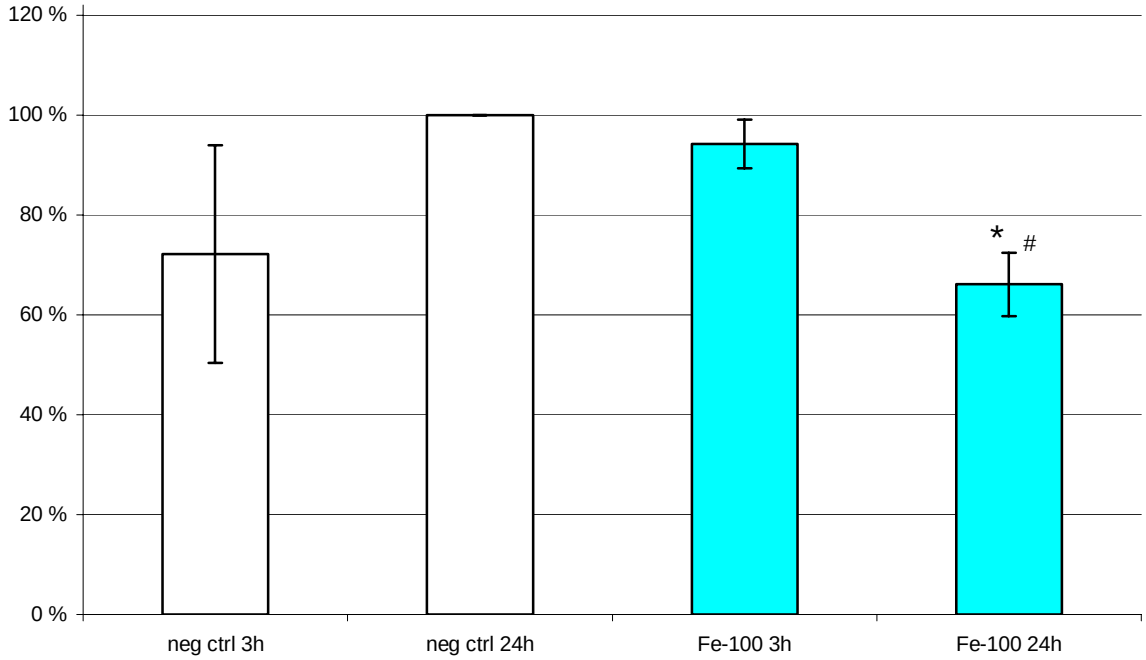
A–4.2. Ferrous sulphate 100 µM (see legend p. 52, discussion p. 54)

Slice weights (mg)



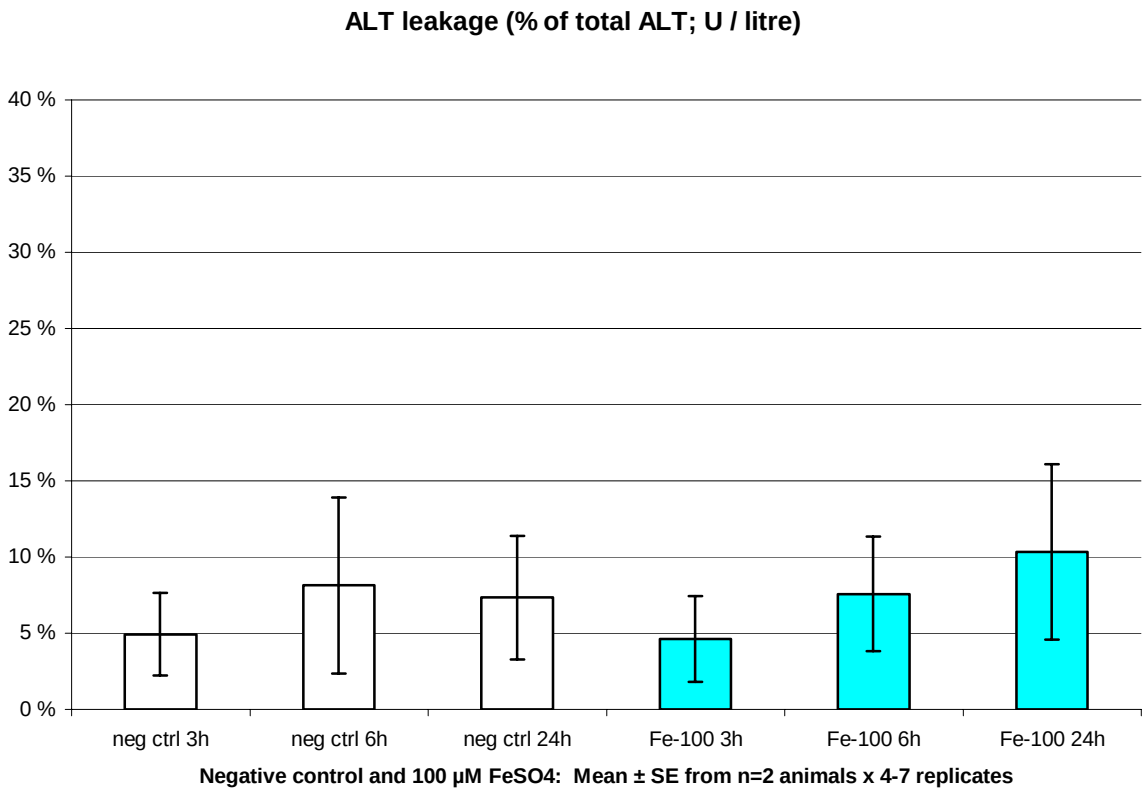
Negative control and 100 µM FeSO4: Mean ± SE from n=2 animals x 4-7 replicates

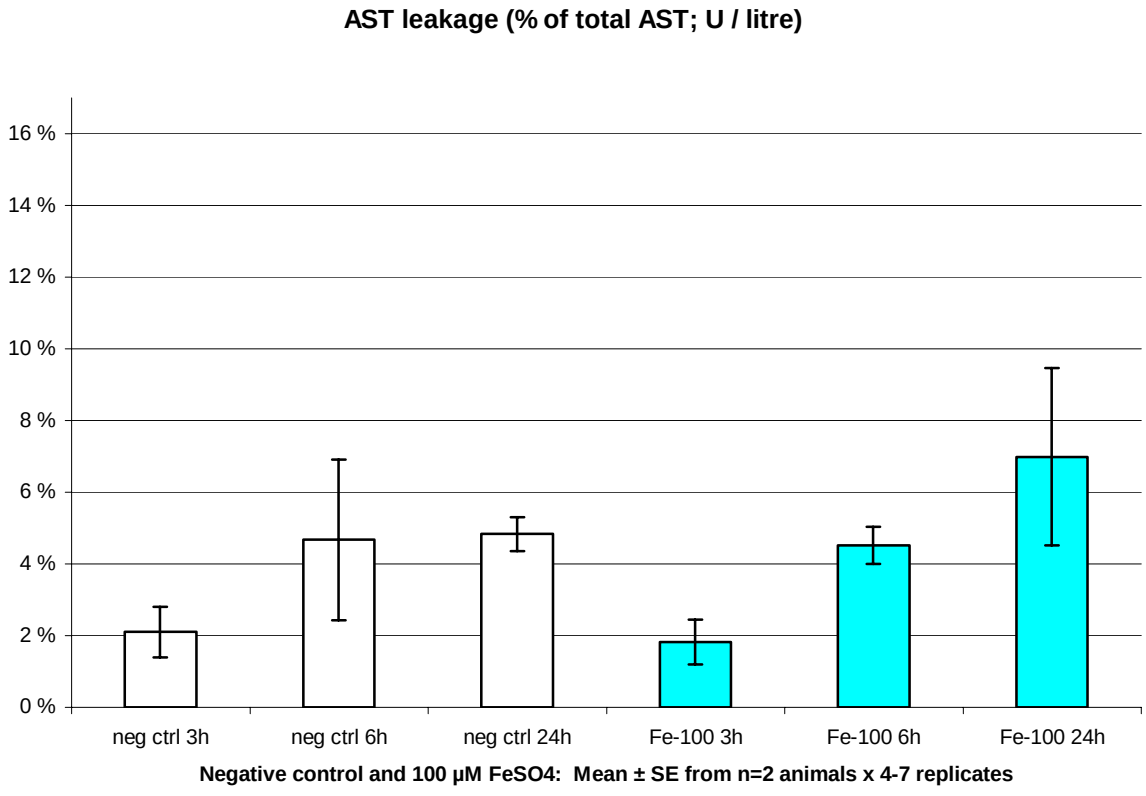
MTT test (% of 24h negative control; OD-570 / mg slice weight)



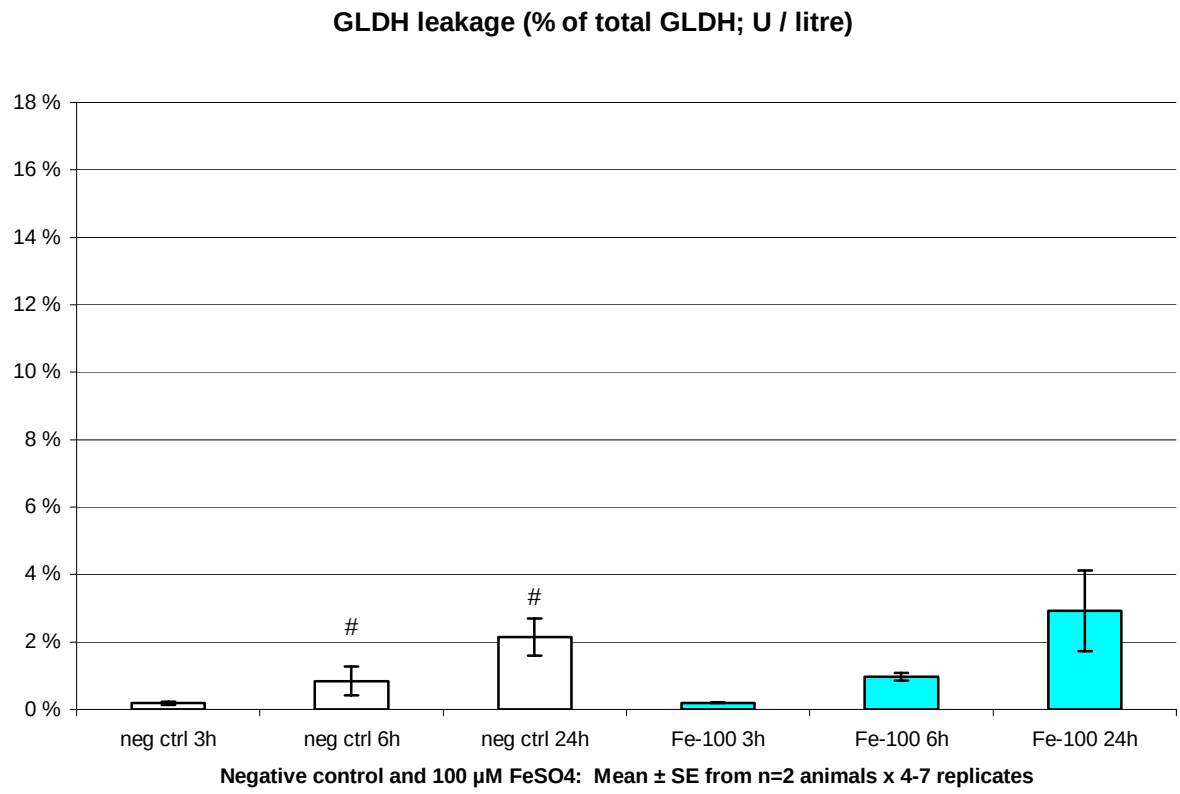
Negative control and 100 µM FeSO4: Mean ± SE from n=2 animals x 2 replicates

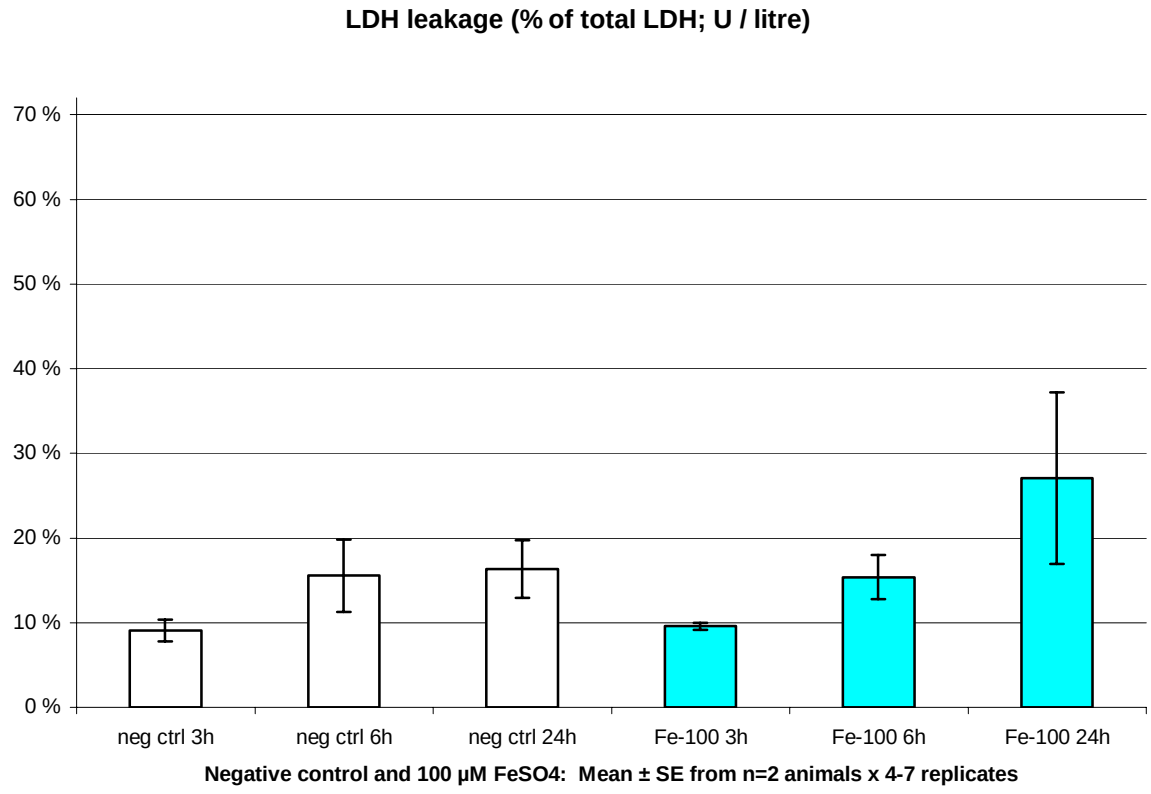
Ferrous sulphate 100 µM (continued; see legend p. 52, discussion p. 54)



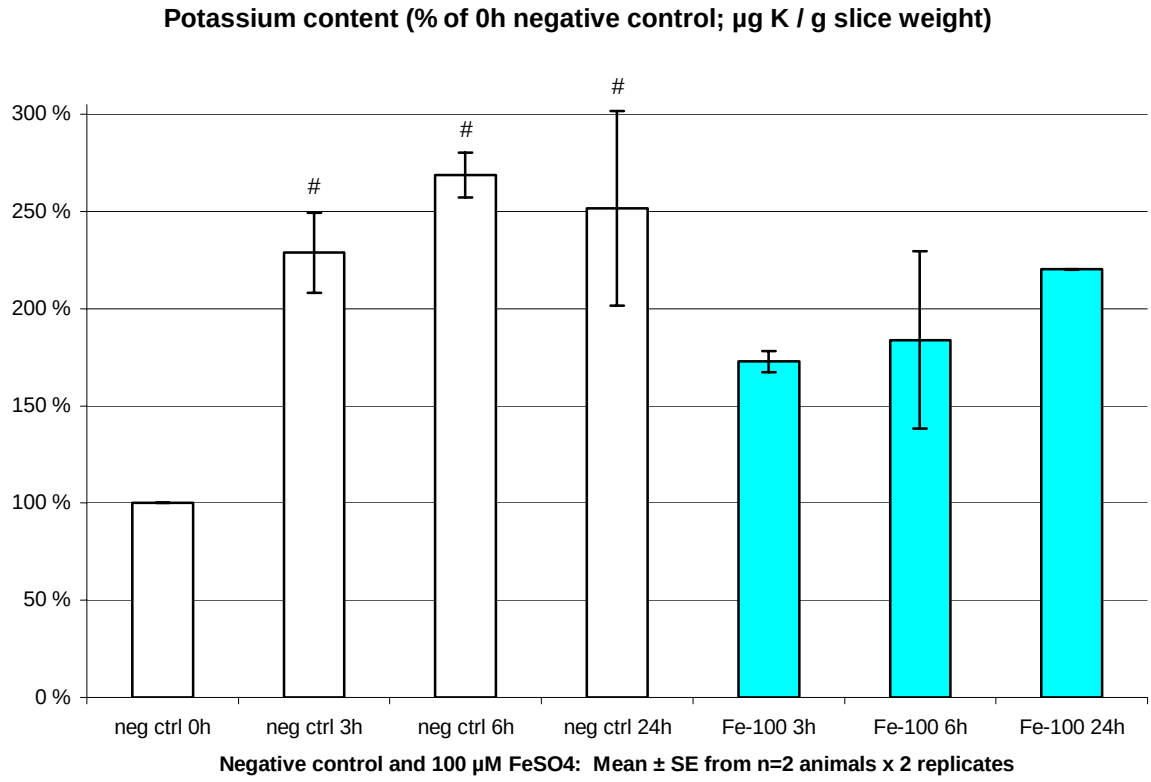


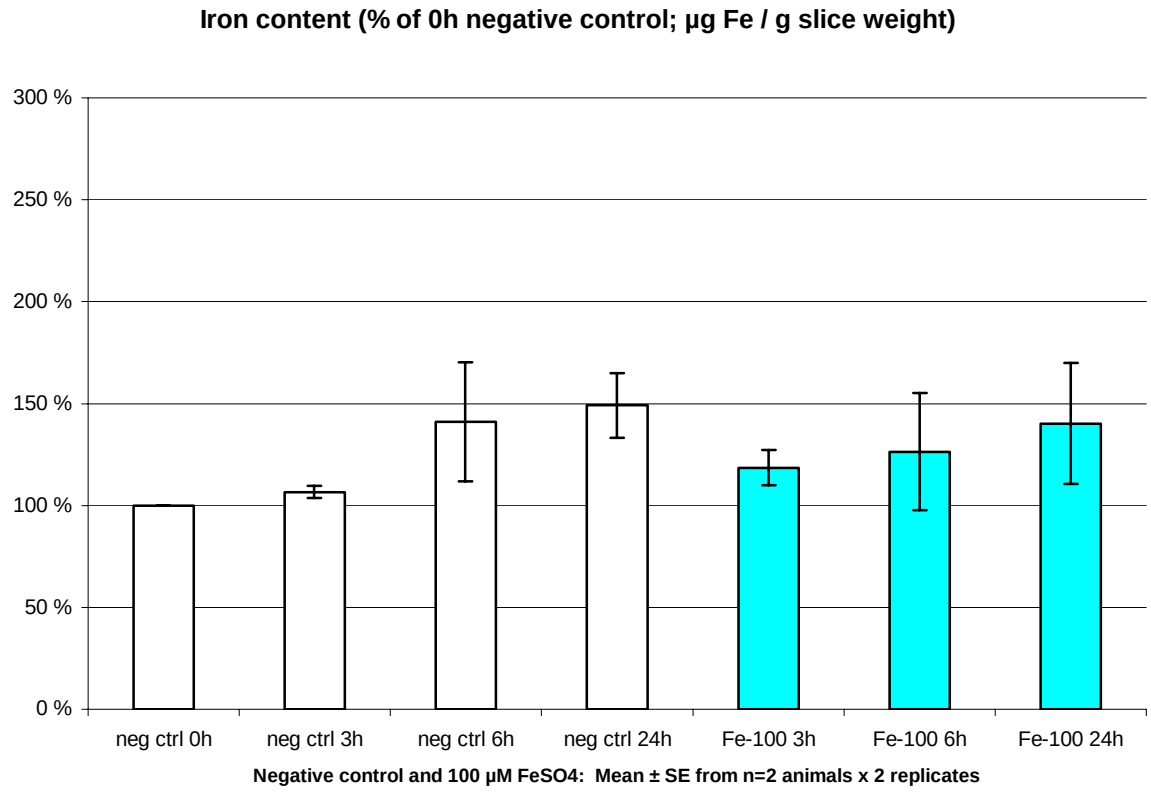
Ferrous sulphate 100 µM (continued; see legend p. 52, discussion p. 54)



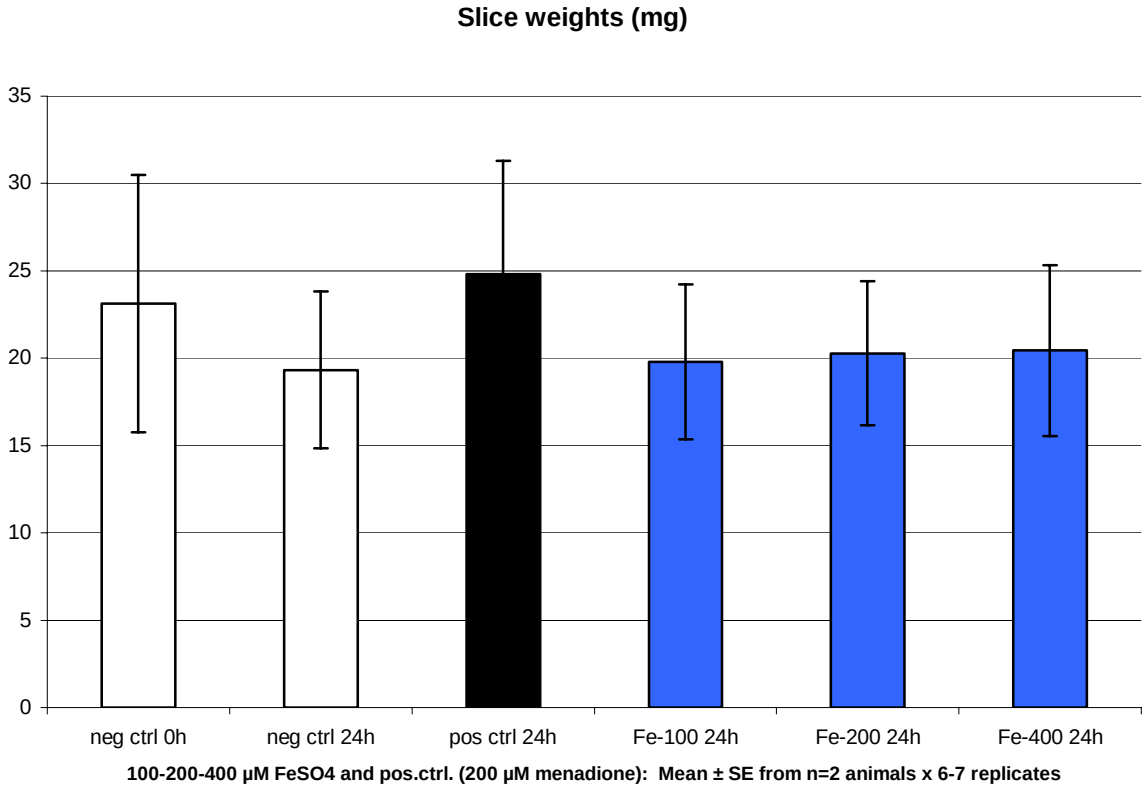


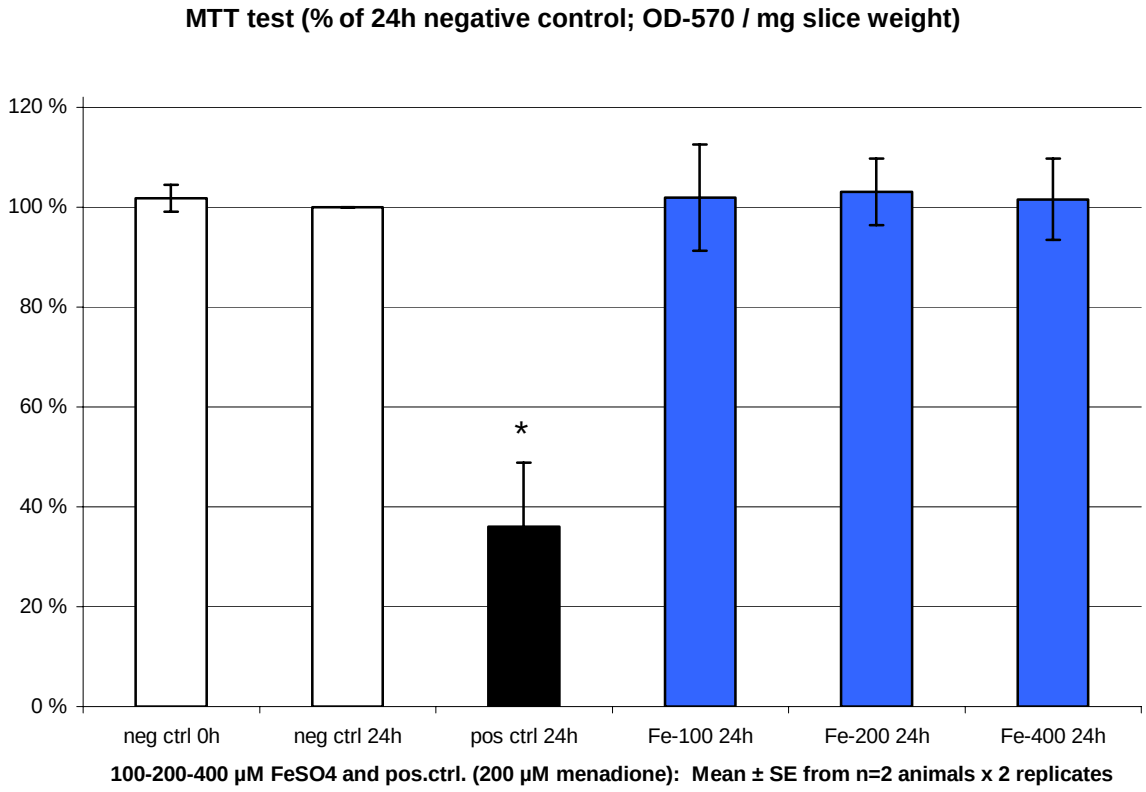
Ferrous sulphate 100 µM (continued; see legend p. 52, discussion p. 54)



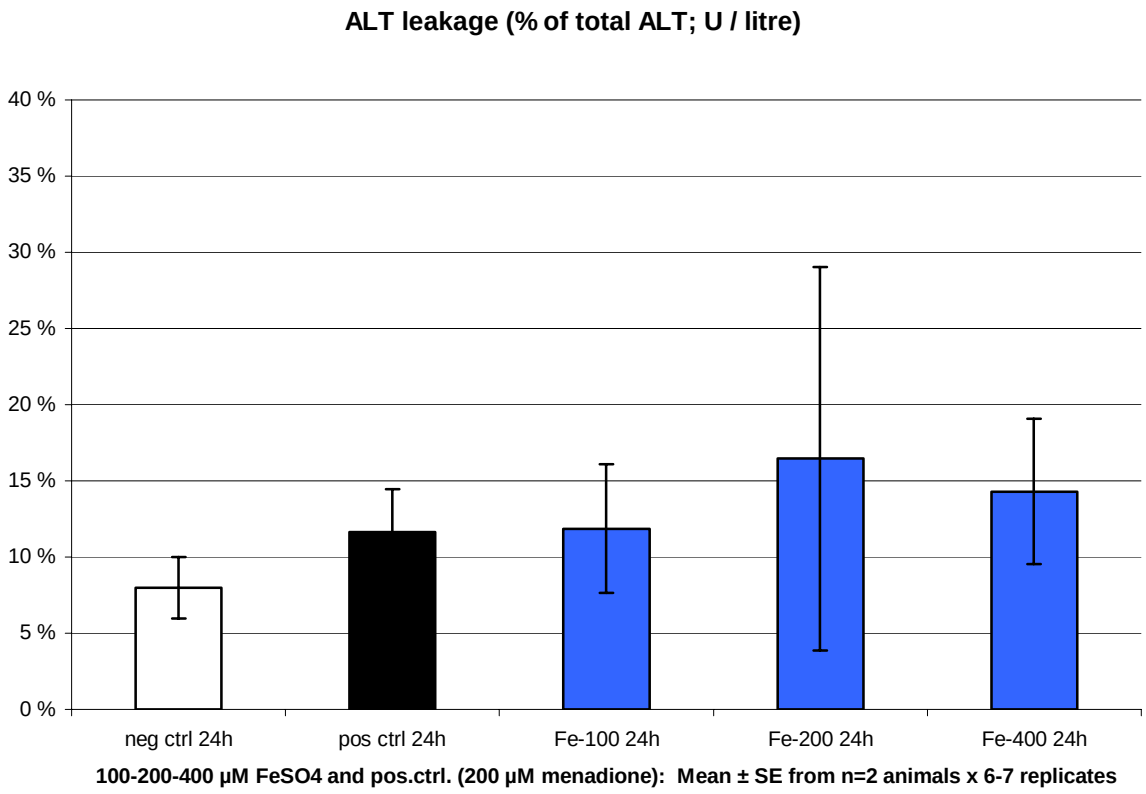


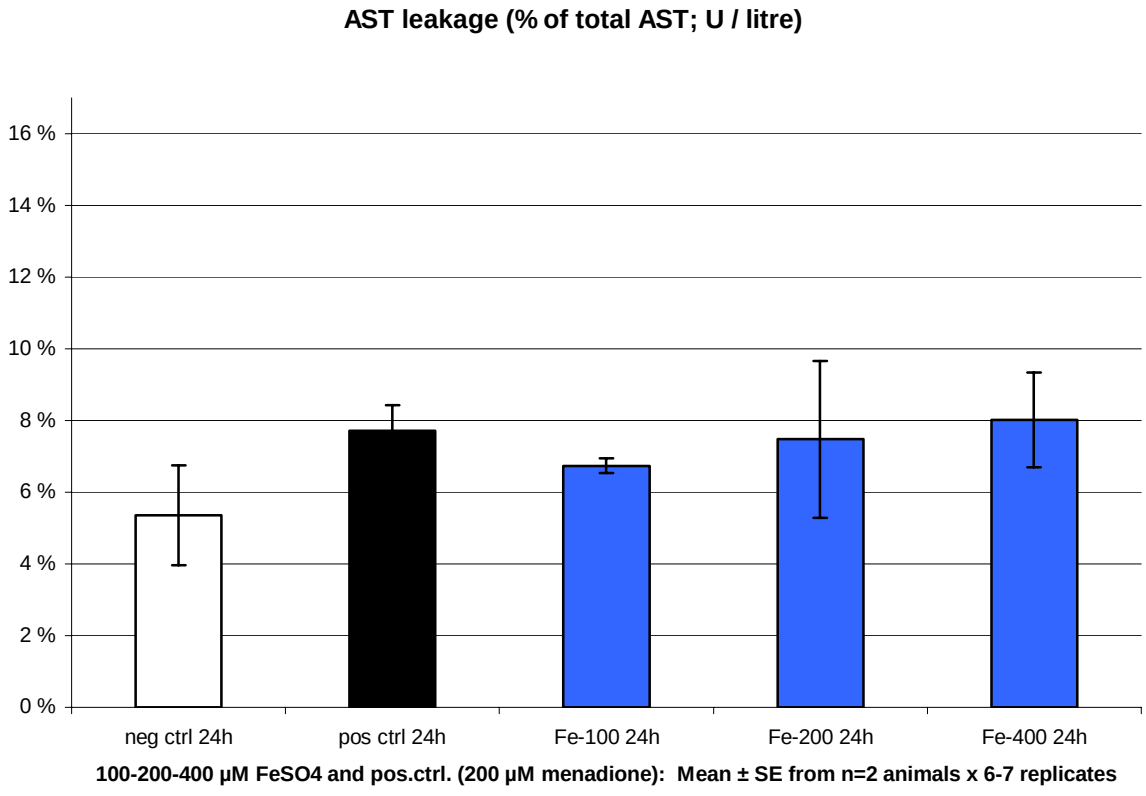
A–4.3. Ferrous sulphate 100, 200, or 400 µM (see legend p. 52, discussion p. 56)



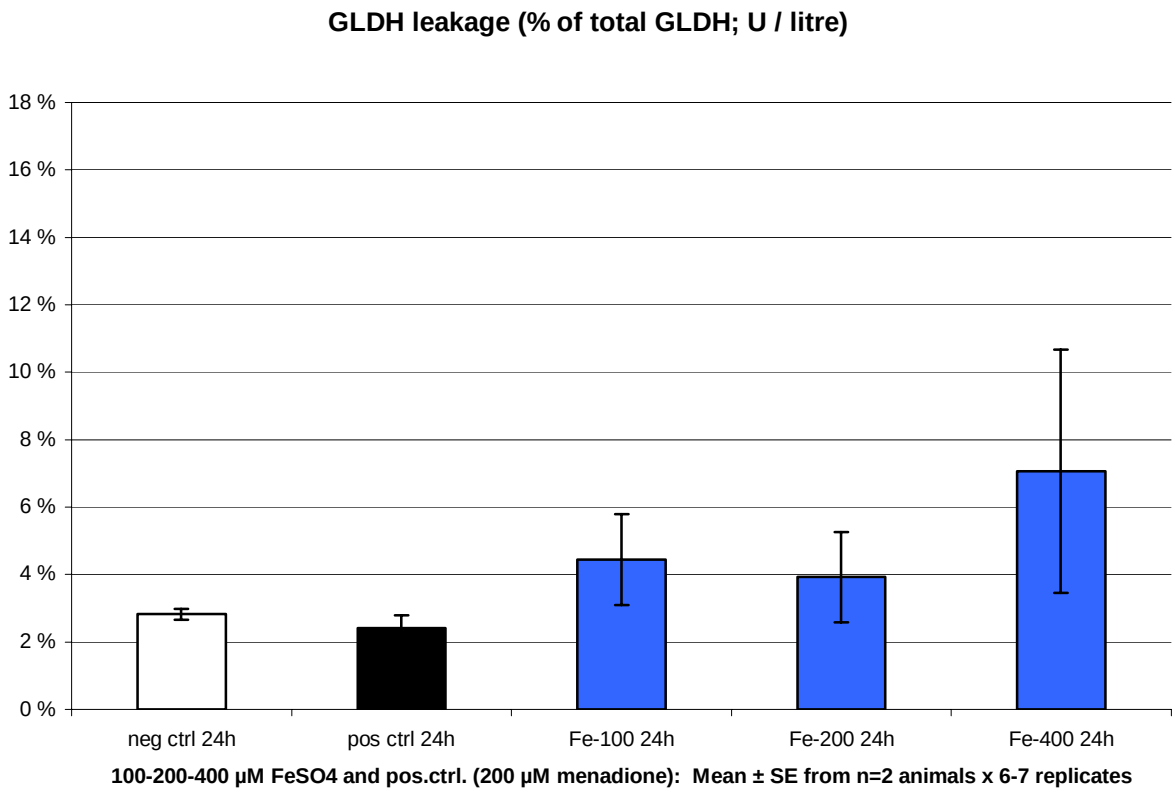


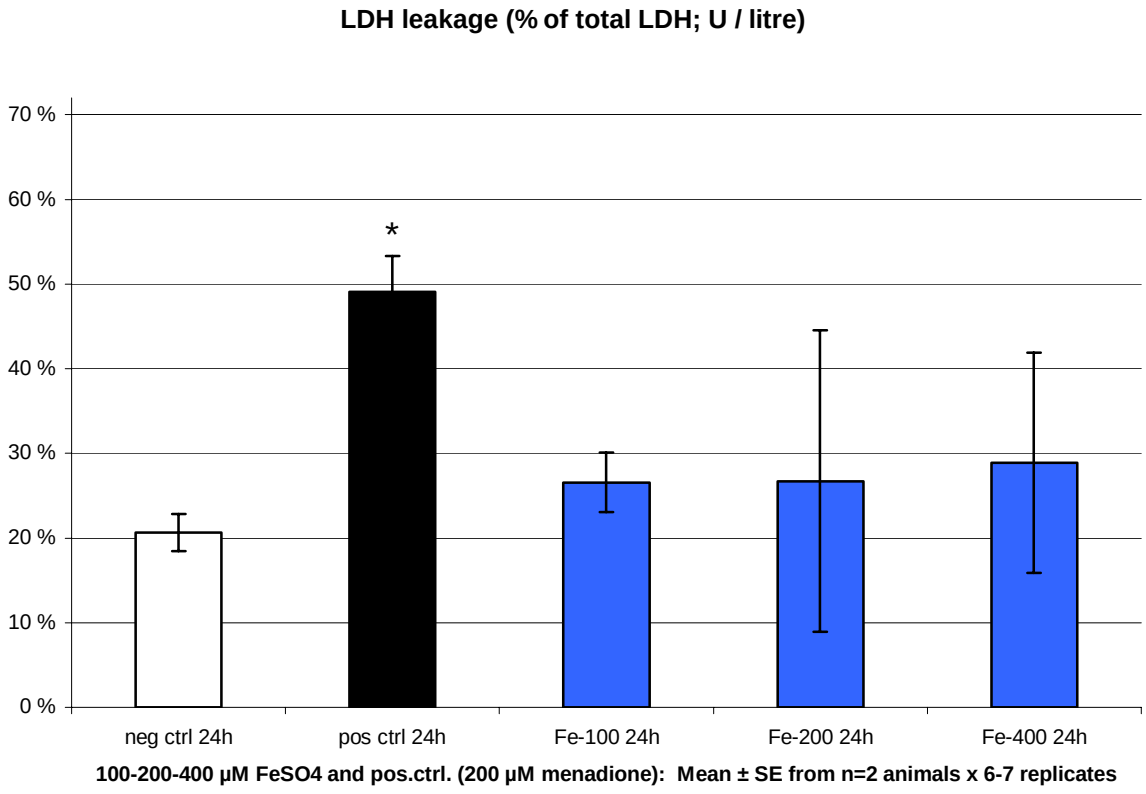
Ferrous sulphate 100, 200, or 400 µM (cont.; legend p. 52, discussion p. 56)





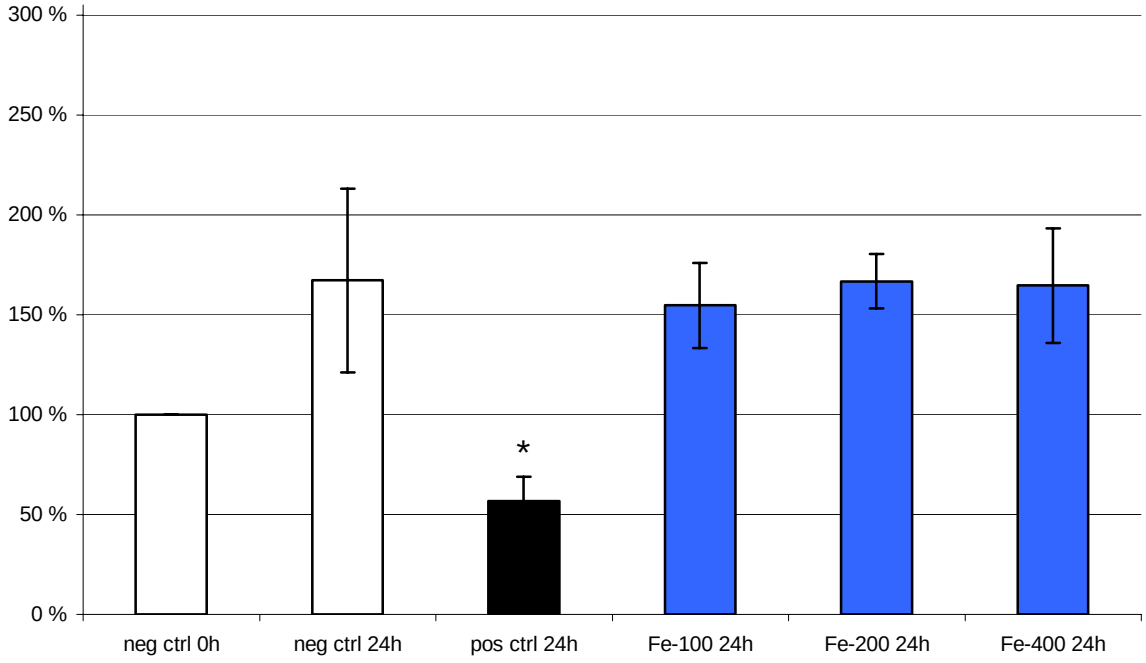
Ferrous sulphate 100, 200, or 400 µM (cont.; legend p. 52, discussion p. 56)





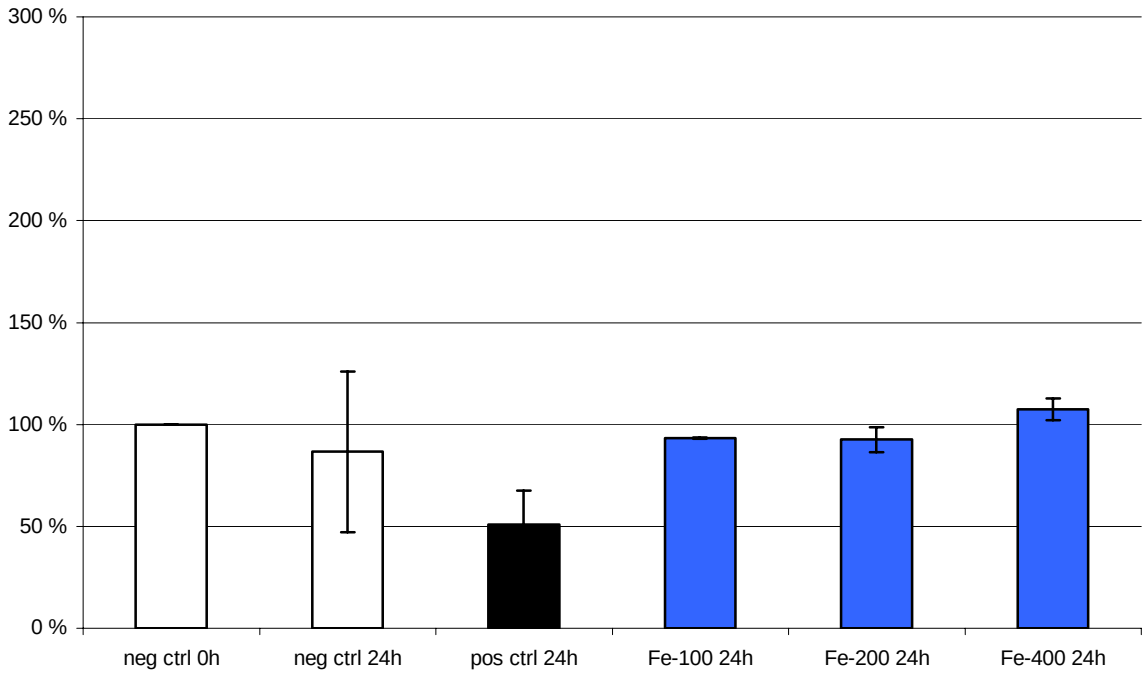
Ferrous sulphate 100, 200, or 400 µM (cont.; legend p. 52, discussion p. 56)

Potassium content (% of 0h negative control; µg K / g slice weight)



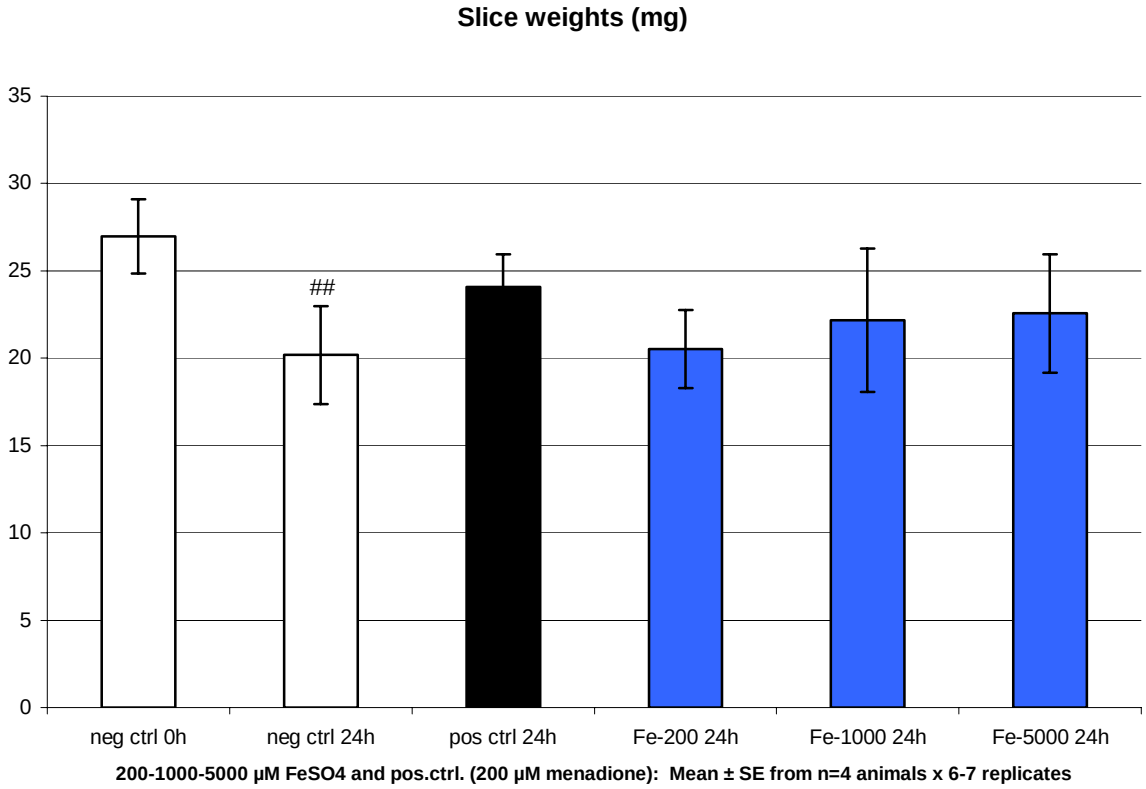
100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione): Mean ± SE from n=2 animals x 2 replicates

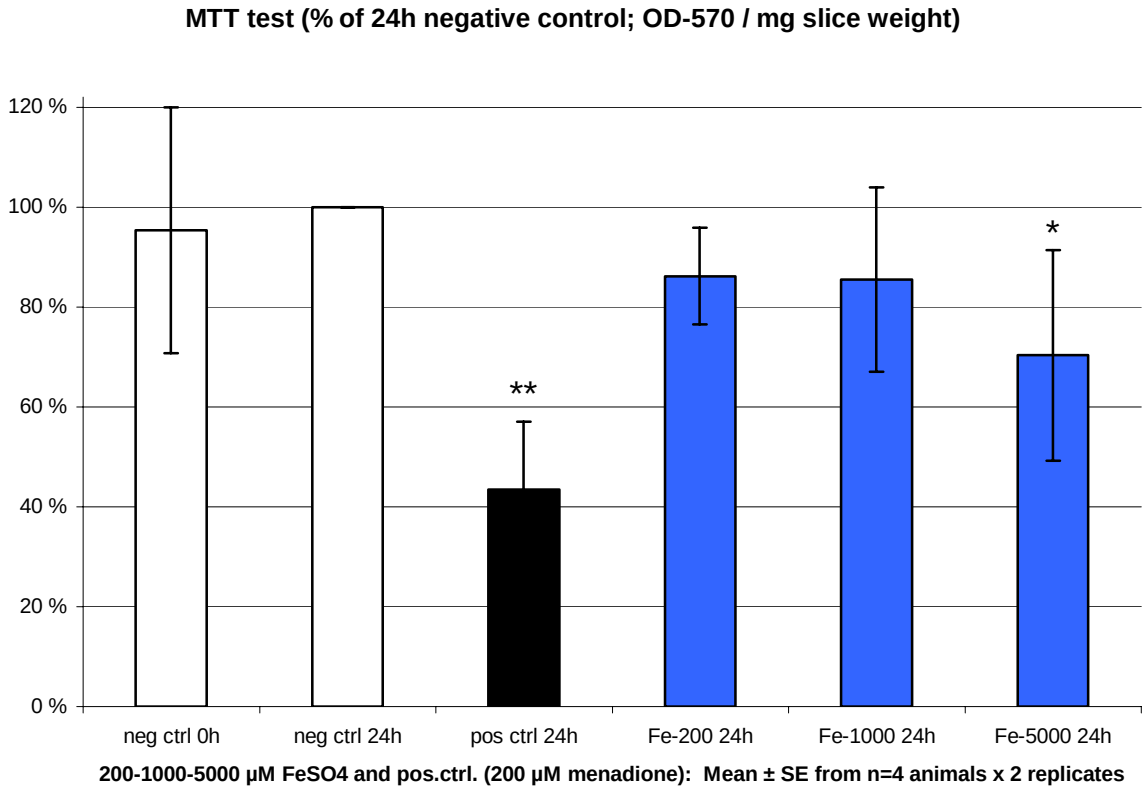
Iron content (% of 0h negative control; µg Fe / g slice weight)



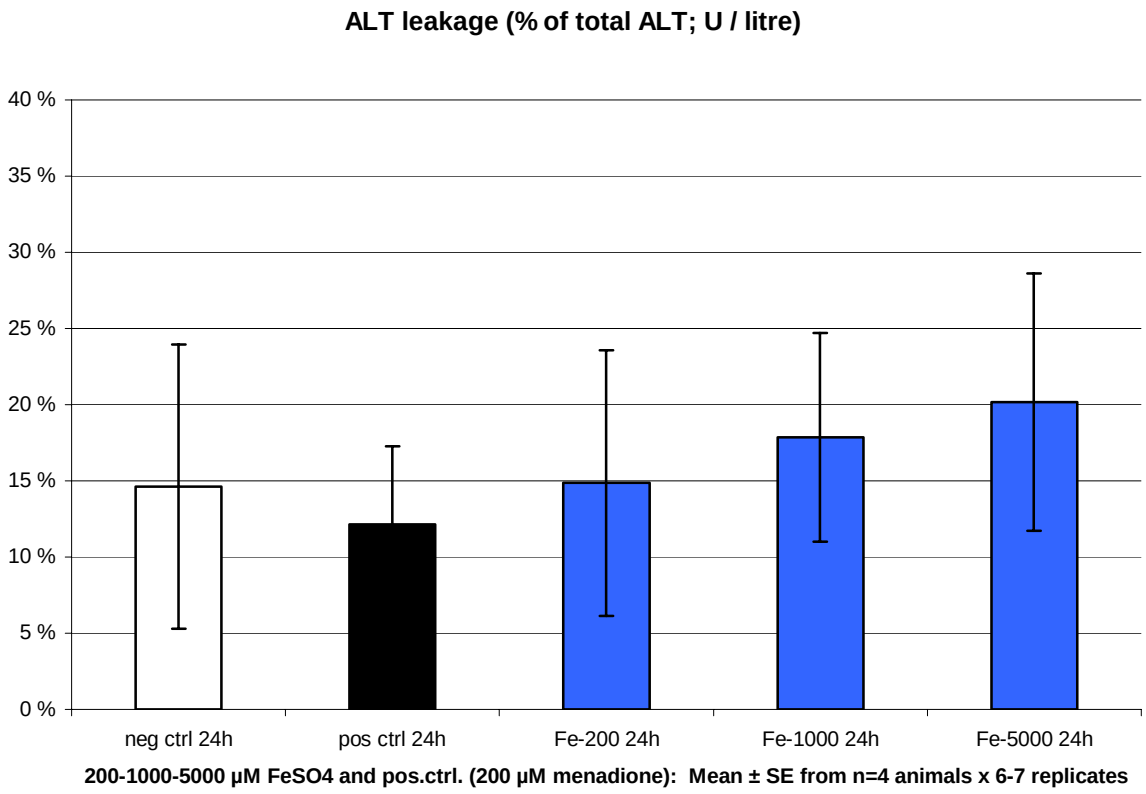
100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione): Mean ± SE from n=2 animals x 2 replicates

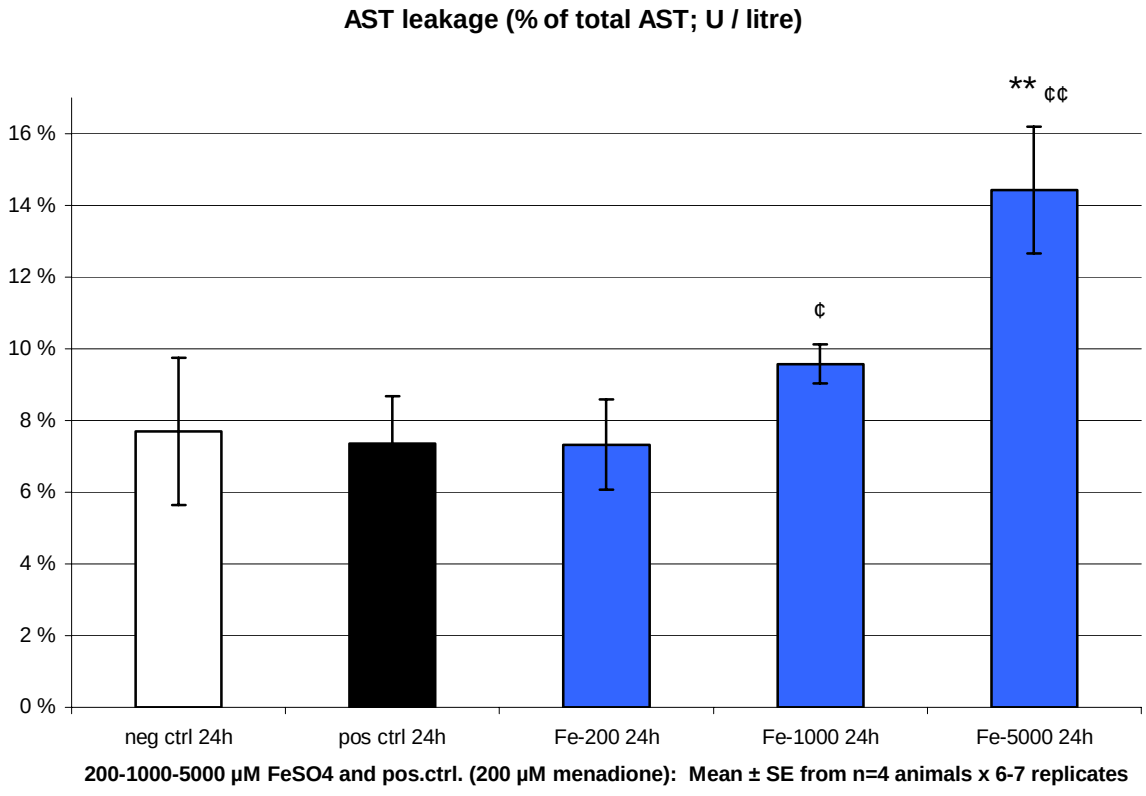
A–4.4. Ferrous sulphate 200, 1000, or 5000 µM (see legend p. 52, discussion p. 57)



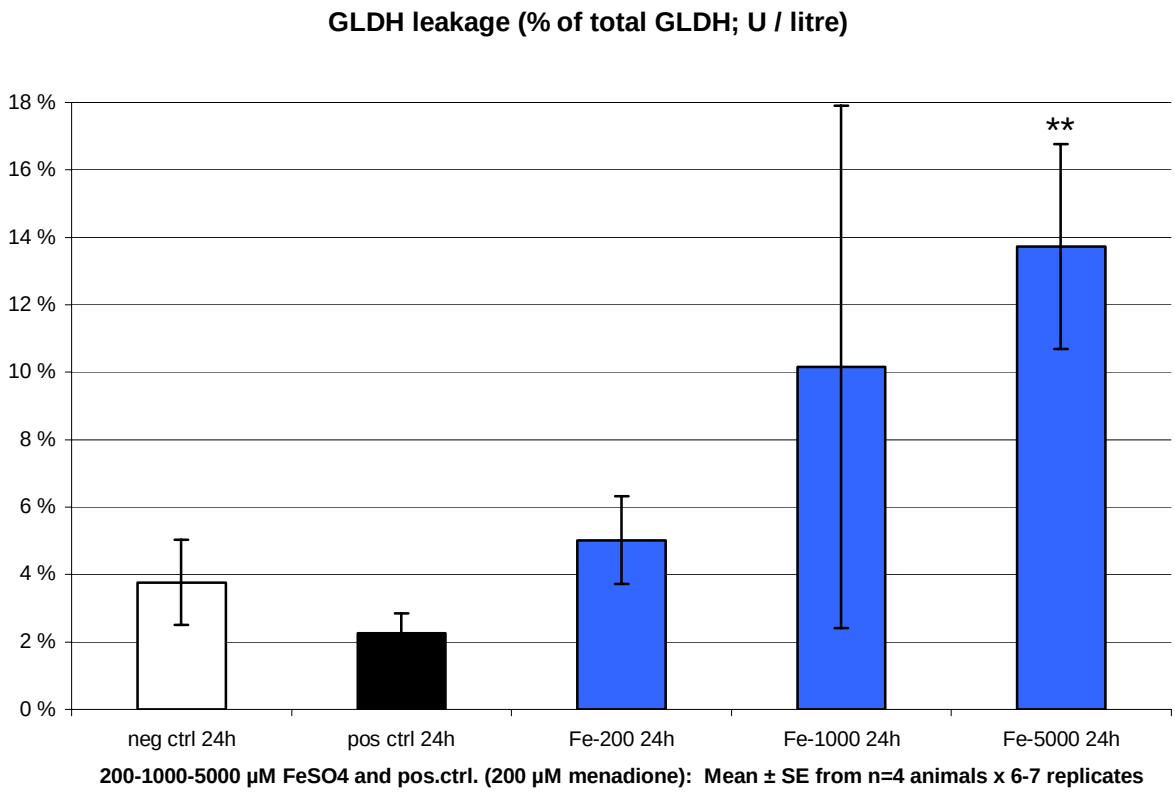


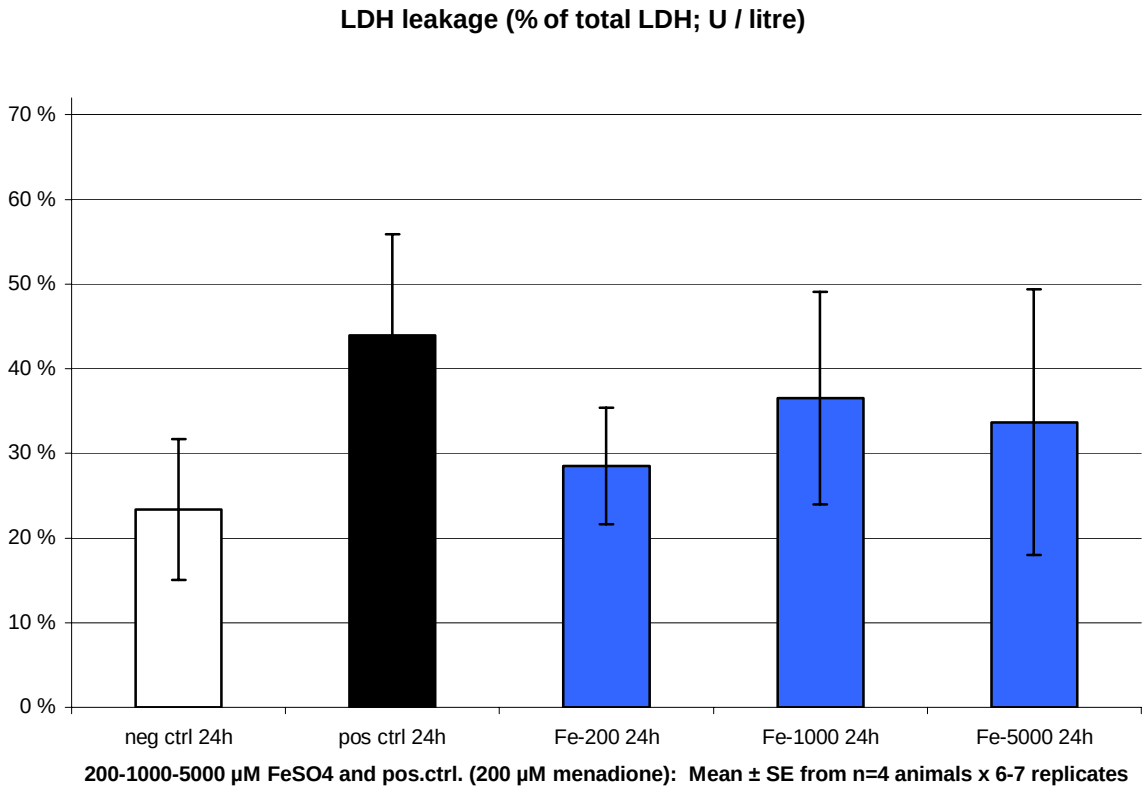
Ferrous sulphate 200, 1000, or 5000 µM (cont.; legend p. 52, discussion p. 57)



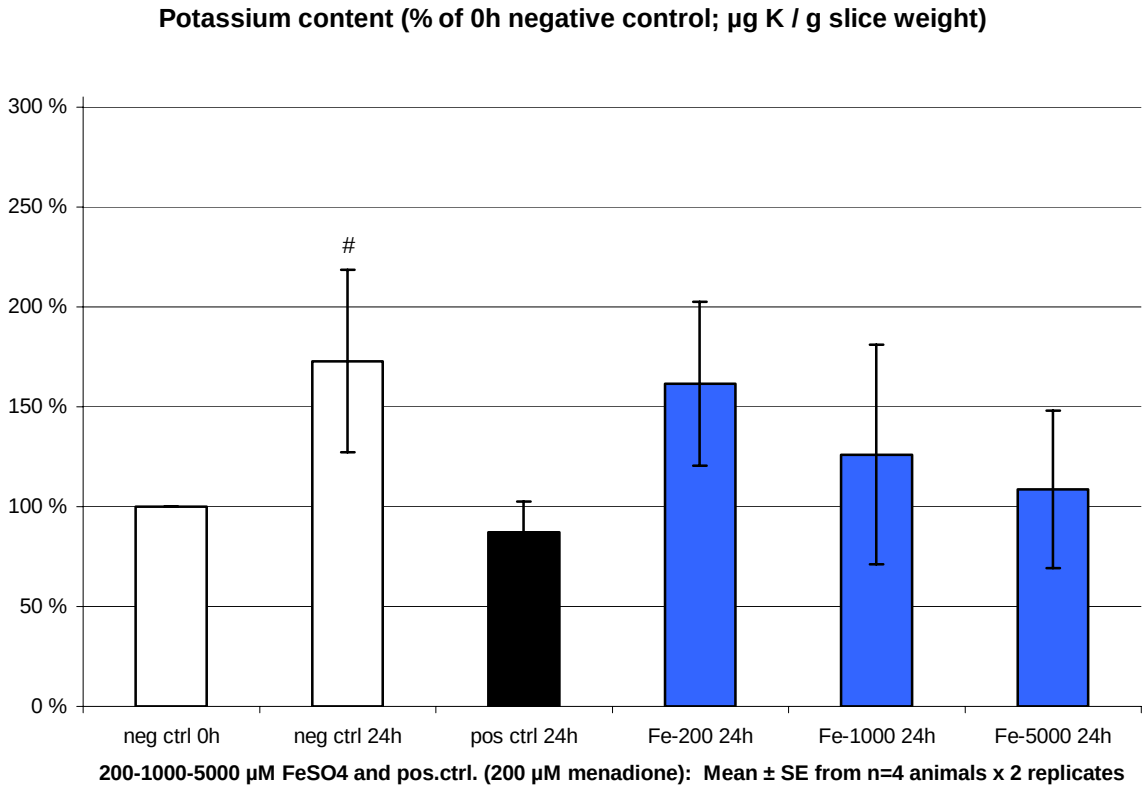


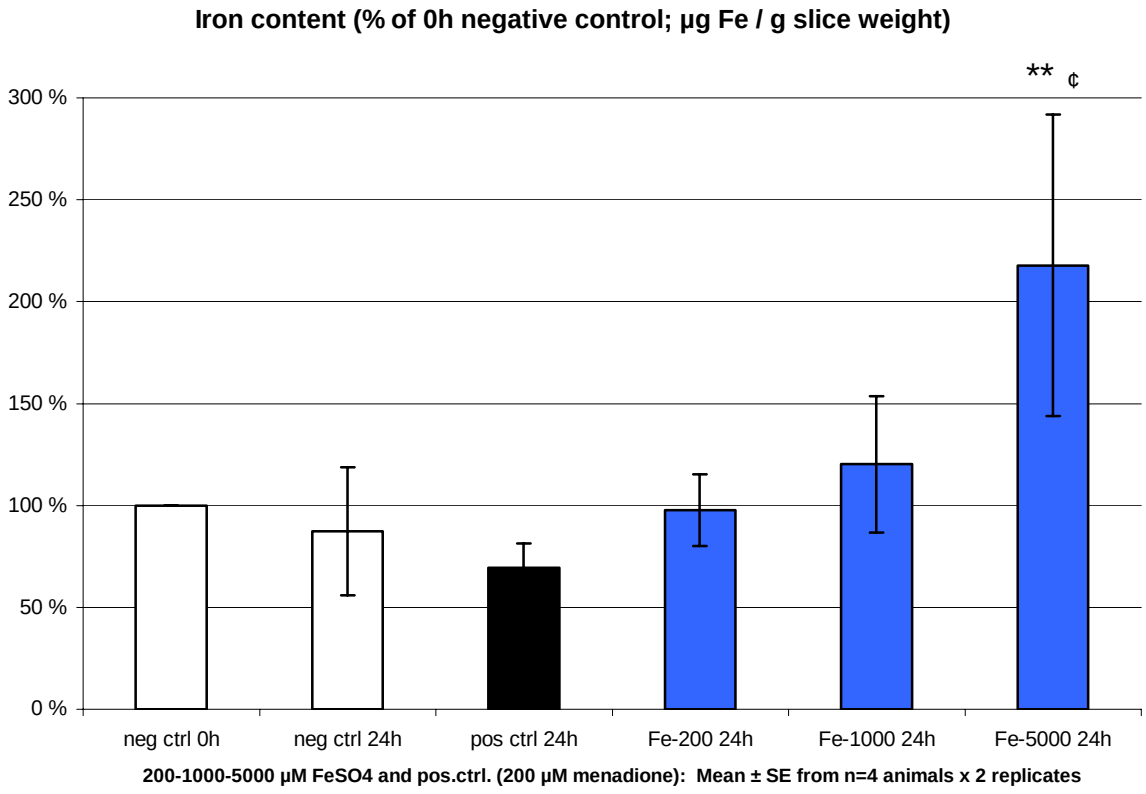
Ferrous sulphate 200, 1000, or 5000 µM (cont.; legend p. 52, discussion p. 57)





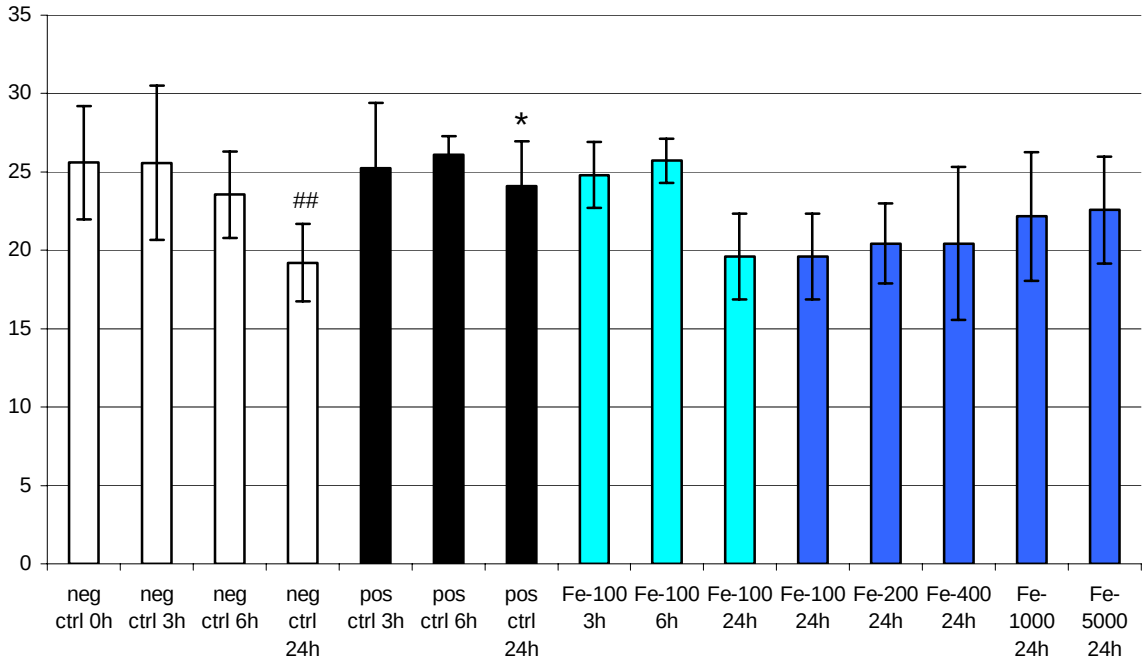
Ferrous sulphate 200, 1000, or 5000 µM (cont.; legend p. 52, discussion p. 57)





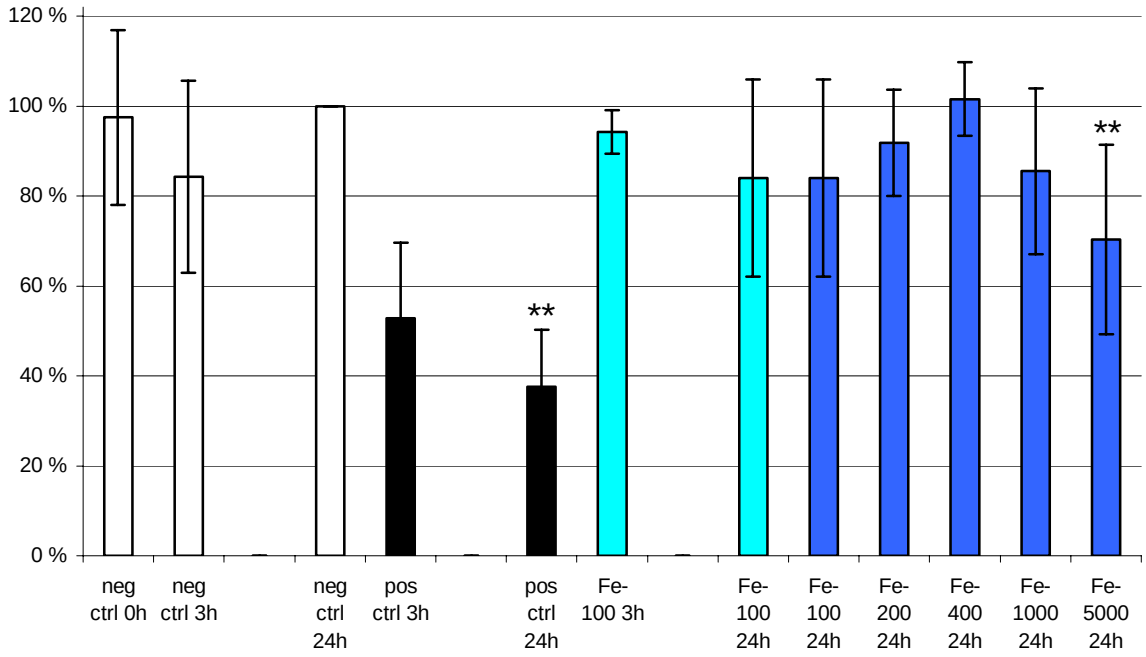
A–4.5. Pooled data from different study designs (see legend p. 52, discussion p. 58)

Slice weights (mg)



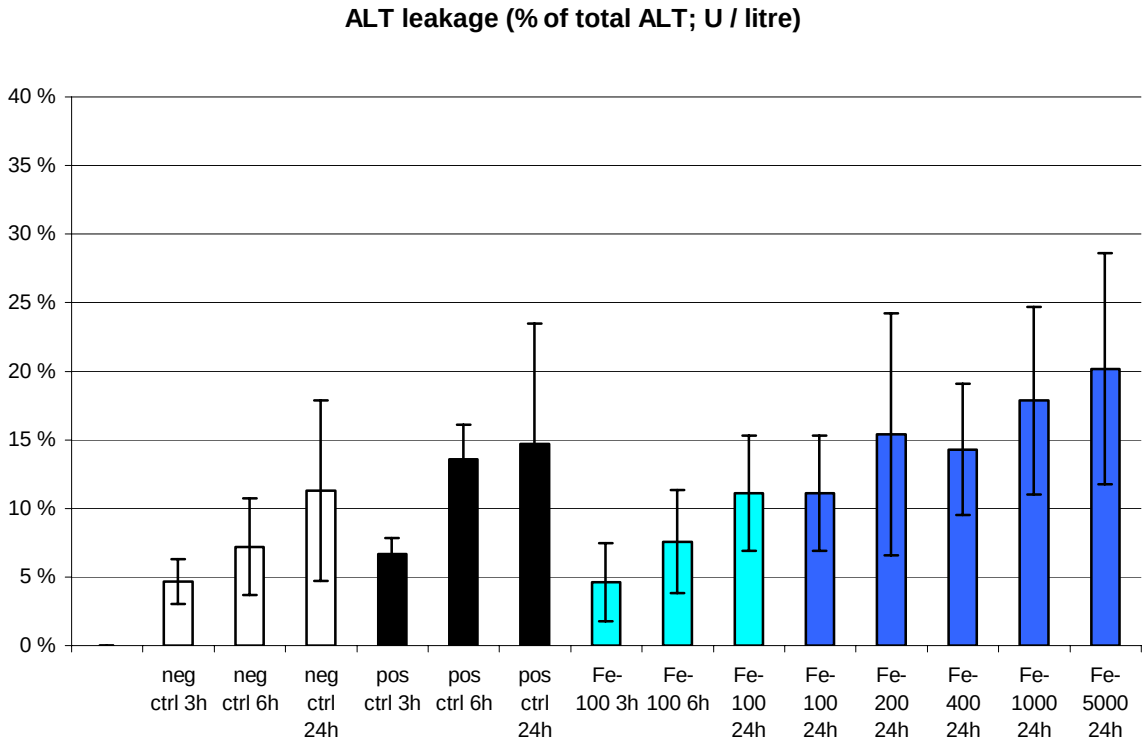
Pooled data: Average ± SE from n=2-10 animals x 4-7 replicates

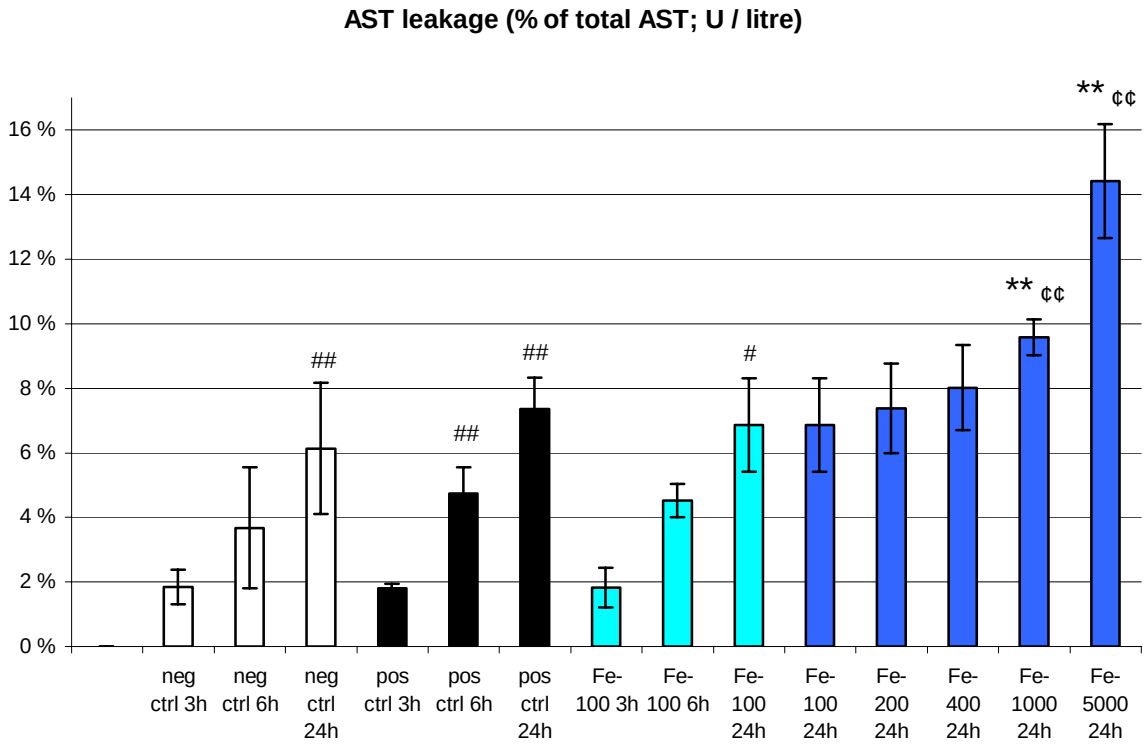
MTT test (% of 24h negative control; OD-570 / mg slice weight)



Pooled data: Average ± SE from n=2-10 animals x 2 replicates

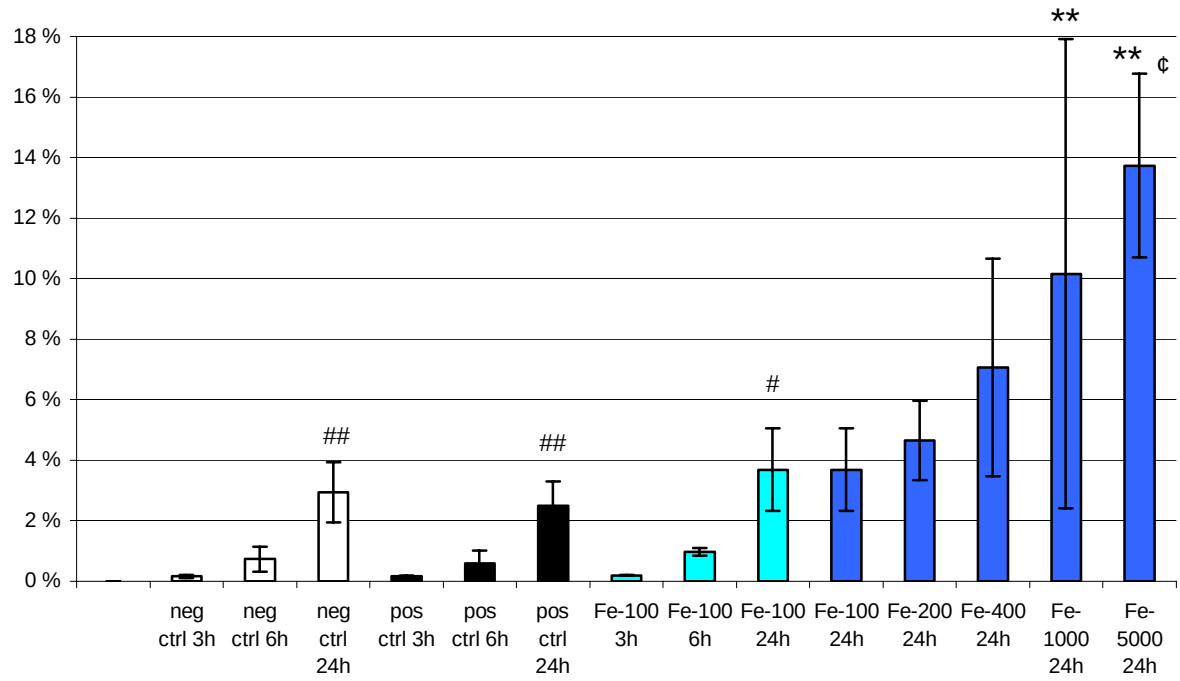
Pooled data from different study designs (cont.; legend p. 52, discussion p. 61)





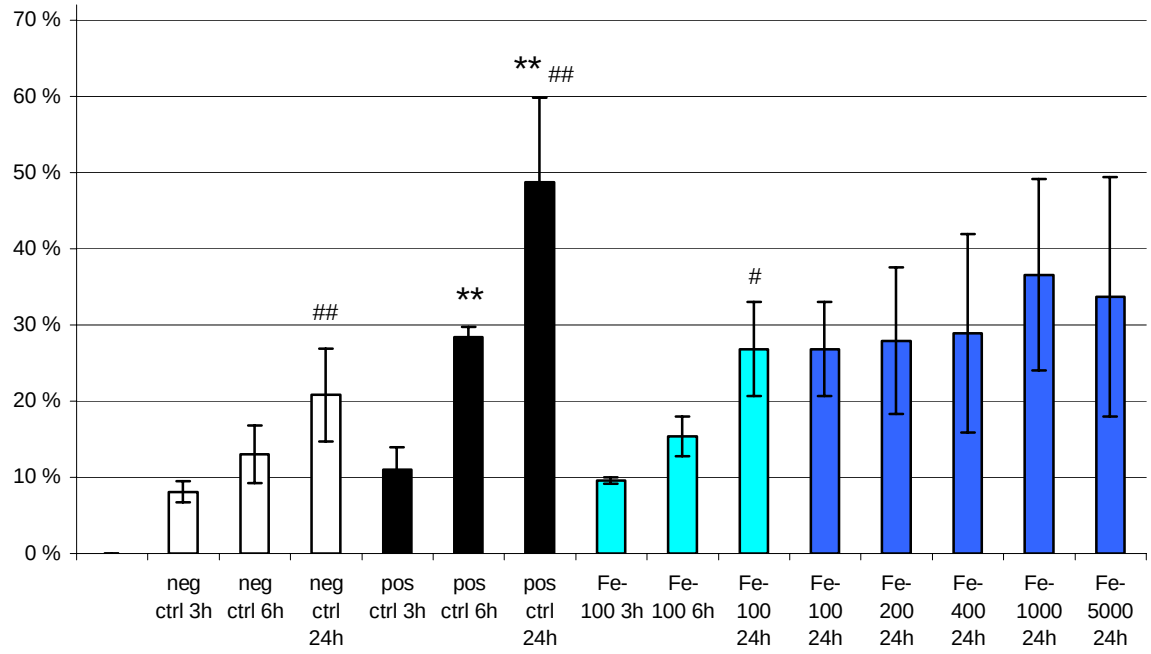
Pooled data from different study designs (cont.; legend p. 52, discussion p. 62)

GLDH leakage (% of total GLDH; U / litre)



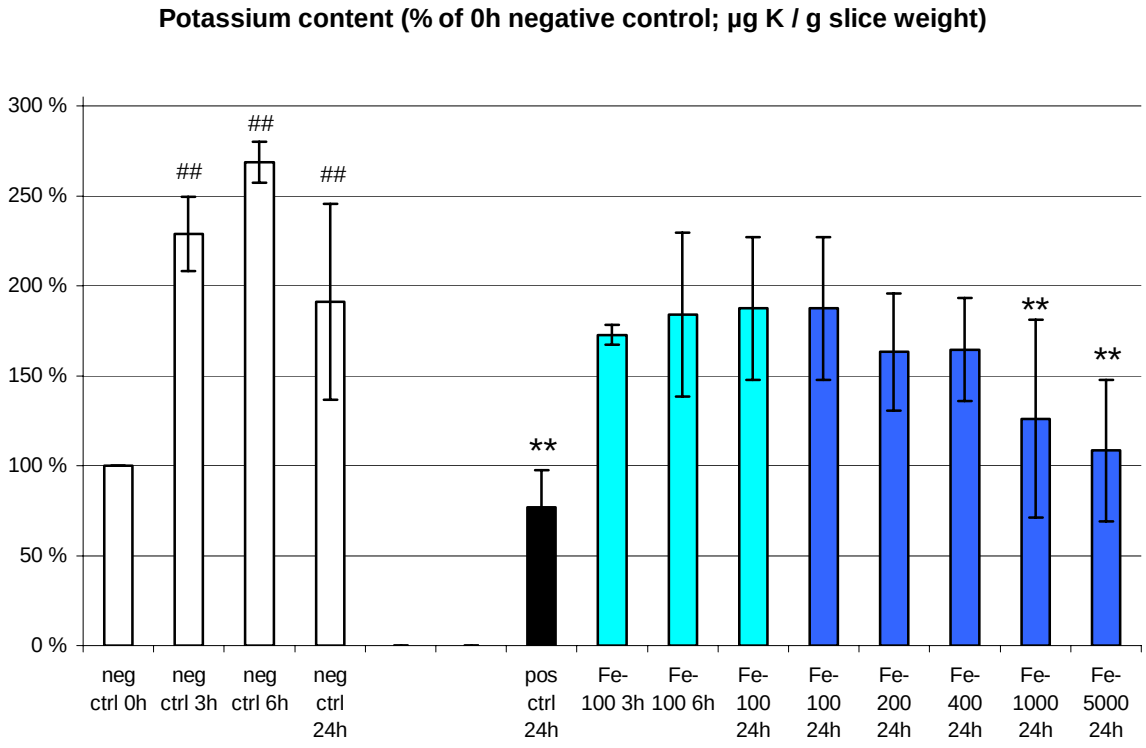
Pooled data: Average ± SE from n=2-10 animals x 4-7 replicates

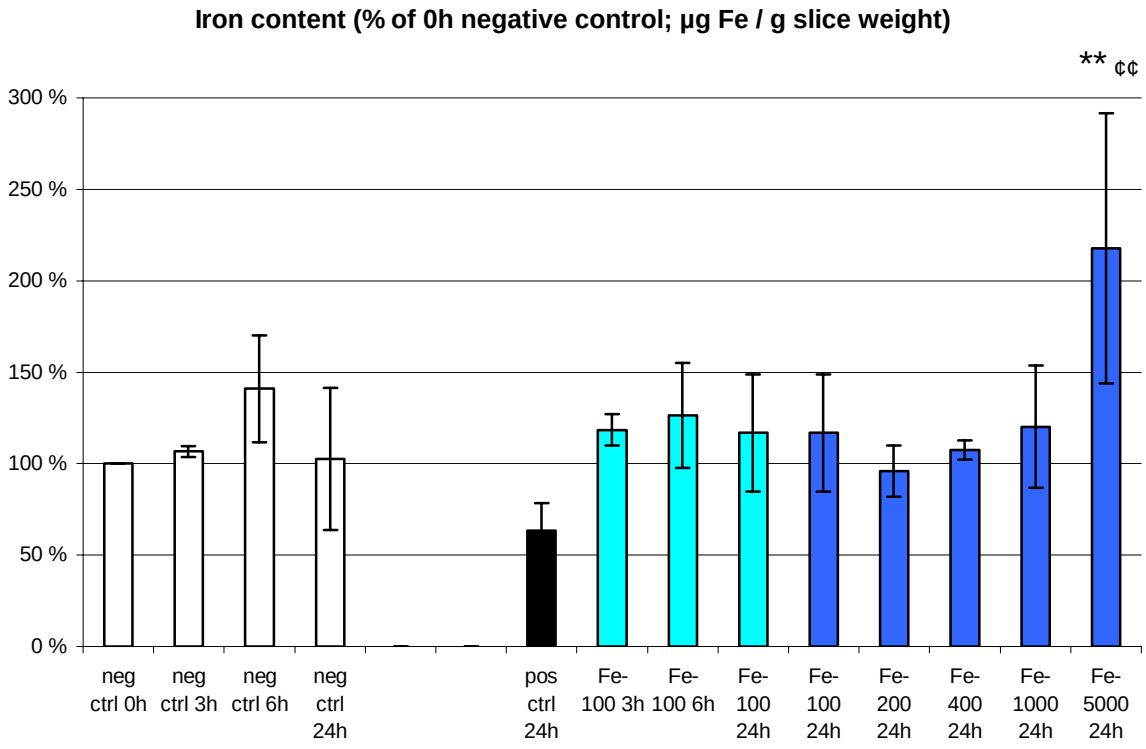
LDH leakage (% of total LDH; U / litre)



Pooled data: Average ± SE from n=2-10 animals x 4-7 replicates

Pooled data from different study designs (cont.; legend p. 52, discussion p. 65)





A–5. RAW DATA

The next pages include raw data and calculations from the following experiments:

- Single-concentration, 3-/6-/24-hour experiments:
  - Experiments EGN-04-00 and EGN-02-01:
    0 or 200 µM menadione for 0, 3, 6, or 24 hours (negative and positive control)
  - Experiments EGN-03-01 and EGN-05-01:
    0 or 100 µM ferrous sulphate for 0, 3, 6, or 24 hours (no positive control)
- Three-concentration, 24-hour experiments:
  - Experiments EGN-06-01 and EGN-07-01:
    0, 100, 200, or 400 µM ferrous sulphate for 24 hours (pos. ctrl.: 200 µM menadione)
  - Experiments EGN-09-01 through EGN-12-01:
    0, 200, 1000, or 5000 µM ferrous sulphate for 24 h (pos. ctrl.: 200 µM menadione)

The data include:

- Slice weights (mg)
- ASAT leakage (% of total ASAT; U / litre) **
- ALAT leakage (% of total ALAT; U / litre)
- GLDH leakage (% of total GLDH; U / litre) **
- LDH leakage (% of total LDH; U / litre) **
- MTT test (% of 24h negative control; OD-570 / mg slice weight) ††
- Potassium content (% of 0h negative control; µg K / g slice weight) ‡‡
- Iron content (% of 0h negative control; µg Fe / g slice weight)

** Enzyme activity (U/litre) was measured in slice incubation medium and calculated as % of total enzyme (sum of enzyme in medium and enzyme remaining in slice).

†† Optical density of formazan blue (absorbency at 570 nm) was measured in isopropanol extract of slice after treatment with MTT, adjusted for slice weight, and calculated as % of the result of negative controls in the same experiment at 24 hours.

‡‡ Metal concentration (ppm) was measured in slice digested in acids, adjusted for slice weight, and calculated as % of the result of negative controls in the same experiment at 0 hours.

EGN-04-00: Effects of exposure time of menadione on rat liver slices

Groups: Group A (menadione 200 uM / DMSO 1%)
Group B (DMSO 1%) B1-B5 (0h-control) - not treated

Slice weights (mg)

time	sample	weight
3h	A6	22,0
	A7	25,8
	A8	19,9
	A9	21,0
	A10	22,7
	mean	22,3
	SD	2,2
6h	A11	28,8
	A12	22,6
	A13	22,0
	A14	26,0
	A15	27,0
	mean	25,3
	SD	2,9
24h	A16	21,4
	A17	21,1
	A18	22,6
	A19	22,6
	A20	23,4
	mean	22,2
	SD	0,9
0h	B1	21,0
	B2	21,7
	B3	24,2
	B4	26,1
	B5	19,2
	mean	22,4
	SD	2,7
3h	B6	22,2
	B7	22,0
	B8	19,0
	B9	19,4
	B10	26,1
	mean	21,7
	SD	2,8
6h	B11	18,1
	B12	22,8
	B13	23,3
	B14	17,8
	B15	23,2
	mean	21,0
	SD	2,8
24h	B16	14,3
	B17	15,5
	B18	18,1
	B19	17,0
	B20	19,9
	mean	17,0
	SD	2,2

comments

Regarding all slices:
Incubator heat was not turned on until 2,5 hours before pre-incubation, so temperature was around 3-5 degrees too low during pre-incubation, and 1-3 degrees too low for the first 2-3 hours of incubation.
(Data is available from trials determining temperature equilibration times for incubator. 36-37 degrees C is regarded optimal temperature.)
At the end of treatment (24h) the heat sensor indicated almost 50 degrees C, but it is not known for how long the slices were treated under too hot conditions.
24h-treated slices in group A looked like cooked ham.

EGN-04-00: Effects of exposure time of menadione on rat liver slices

Groups: Group A (menadione 200 uM / DMSO 1%)
Group B (DMSO 1%) B1-B5 (0h-control) - not treated

MTT test (OD-570); raw data minus background					**Abs.570nm / mg slice wet weight**
time	sample	OD-570	versus weight	and v. 24-h neg. (%)	slice
3h	A8-1	0,599	0,030	58,7 %	
	A8-2	0,608	0,031	59,6 %	59,1 %
	A9-1	0,759	0,036	70,5 %	
	A9-2	0,751	0,036	69,7 %	70,1 %
	mean	0,679	0,033	64,6 %	64,6 %
	SD	0,088	0,003	6,4 %	7,8 %
24h	A18-1	0,167	0,007	14,4 %	
	A18-2	0,162	0,007	14,0 %	14,2 %
	A19-1	0,344	0,015	29,7 %	
	A19-2	0,340	0,015	29,3 %	29,5 %
	mean	0,253	0,011	21,9 %	21,9 %
	SD	0,103	0,005	8,8 %	10,8 %
3h	B8-1	0,893	0,047	91,7 %	
	B8-2	0,933	0,049	95,8 %	93,7 %
	B9-1	1,231	0,063	123,7 %	
	B9-2	1,233	0,064	123,9 %	123,8 %
	mean	1,073	0,056	108,8 %	108,8 %
	SD	0,185	0,009	17,5 %	21,3 %
24h	B18-1	1,013	0,056	109,1 %	
	B18-2	1,064	0,059	114,6 %	111,9 %
	B19-1	0,753	0,044	86,4 %	
	B19-2	0,783	0,046	89,8 %	88,1 %
	mean	0,903	0,051	100,0 %	100,0 %
	SD	0,158	0,007	14,0 %	16,8 %

EGN-04-00: Effects of exposure time of menadione on rat liver slices

Groups: Group A (menadione 200 uM / DMSO 1%)
Group B (DMSO 1%) B1-B5 (0h-control) - not treated

background: medium		2	0	0,1	48
Culture media enzyme activities (U/L); raw data					
time	sample	ASAT	ALAT	GLDH	LDH
3h	A6	40	18	1,8	502
	A7	48	21	1,7	626
	A8	30	12	1,4	395
	A9	33	15	1,3	387
	A10	29	13	1,0	382
	mean	36,0	15,8	1,4	458
	SD	8,0	3,7	0,3	106
6h	A11	108	38	3,8	1 330
	A12	59	21	2,6	722
	A13	77	23	4,9	856
	A14	59	21	2,5	707
	A15	83	31	3,1	941
	mean	77,2	26,8	3,4	911
	SD	20,3	7,5	1,0	253
24h	A16	77	15	8,7	1 067
	A17	100	17	14,7	1 313
	A18	78	15	14,5	1 169
	A19	49	9	10,3	1 229
	A20	71	10	13,5	1 169
	mean	75,0	13,2	12,3	1 189
	SD	18,2	3,5	2,7	90
0h					
3h	B6	21	9	1,3	303
	B7	35	19	1,0	436
	B8	27	15	1,2	339
	B9	27	13	0,8	301
	B10	27	15	1,3	325
	mean	27,4	14,2	1,1	341
	SD	5,0	3,6	0,2	56
6h	B11	34	18	2,3	363
	B12	47	23	3,1	548
	B13	56	23	4,1	584
	B14	30	16	1,9	377
	B15	42	18	4,8	429
	mean	41,8	19,6	3,2	460
	SD	10,4	3,2	1,2	100
24h	B16	44	14	8,4	346
	B17	57	13	12,9	439
	B18	51	14	14,3	394
	B19	72	16	20,4	577
	B20	94	19	29,7	703
	mean	63,6	15,2	17,1	492
	SD	19,9	2,4	8,2	146

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
3h	A6				
	A7				
	A8				
	A9				
	A10	132	14	73,4	221
	mean	132,0	14,0	73,4	221
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	A11				
	A12				
	A13	103	11	66,4	132
	A14	133	16	84,1	214
	A15	142	16	87,7	183
	mean	126,0	14,3	79,4	176
	SD	20,4	2,9	11,4	41
24h	A16				
	A17				
	A18				
	A19				
	A20	67	2	19,9	43
	mean	67,0	2,0	19,9	43
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0h	B1				
	B2				
	B3	133	19	87,0	327
	B4	144	17	86,6	293
	B5	134	15	71,6	244
	mean	137,0	17,0	81,7	288
	SD	6,1	2,0	8,8	42
3h	B6				
	B7				
	B8				
	B9				
	B10	138	20	84,1	295
	mean	138,0	20,0	84,1	295
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	B11				
	B12				
	B13	165	21	98,1	280
	B14	146	19	77,0	237
	B15	172	26	97,7	326
	mean	161,0	22,0	90,9	281
	SD	13,5	3,6	12,1	45
24h	B16				
	B17				
	B18				
	B19				
	B20	127	10	60,0	175
	mean	127,0	10,0	60,0	175
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-02-01: Effects of exposure time of menadione on rat liver slices

Groups: Group A (menadione 200 uM / DMSO 1%)
Group B (DMSO 1%) B1-B5 (0h-control) - not treated

Slice weights (mg)

time	sample	weight	comments
3h	A6	25,9	
	A7	24,1	
	A8	29,8	
	A9	30,1	
	A10	31,0	
	mean	28,2	
	SD	3,0	
6h	A11	27,6	
	A12	24,1	
	A13	31,7	
	A14	23,7	
	A15	27,6	
	mean	26,9	
	SD	3,2	
24h	A16	23,8	
	A17	20,8	
	A18	27,2	
	A19	23,3	
	A20	28,3	
	mean	24,7	
	SD	3,0	
0h	B1	FALSE	slice missing after pre-incubation
	B2	29,4	
	B3	27,9	
	B4	26,5	
	B5	26,1	
	mean	27,5	
	SD	1,5	
3h	B6	23,7	
	B7	22,7	
	B8	23,1	
	B9	23,8	
	B10	25,7	
	mean	23,8	
	SD	1,2	
6h	B11	17,8	
	B12	23,1	
	B13	21,2	
	B14	23,3	
	B15	24,9	
	mean	22,1	
	SD	2,7	
24h	B16	20,5	
	B17	17,3	
	B18	19,2	
	B19	19,9	
	B20	19,6	
	mean	19,3	
	SD	1,2	

EGN-02-01: Effects of exposure time of menadione on rat liver slices

Groups: Group A (menadione 200 uM / DMSO 1%)
Group B (DMSO 1%) B1-B5 (0h-control) - not treated

MTT test (OD-570); raw data minus background					**Abs.570nm / mg slice wet weight**
time	sample	OD-570	versus weight	and v. 24-h neg. (%)	slice
3h	A8-1	0,907	0,030	53,1 %	
	A8-2	0,900	0,030	52,7 %	52,9 %
	A9-1	0,495	0,016	28,7 %	
	A9-2	0,500	0,017	29,0 %	28,9 %
	mean	0,701	0,023	40,9 %	40,9 %
	SD	0,234	0,008	13,9 %	17,0 %
24h	A18-1	0,542	0,020	34,8 %	
	A18-2	0,557	0,020	35,8 %	35,3 %
	A19-1	0,402	0,017	30,1 %	
	A19-2	0,401	0,017	30,0 %	30,1 %
	mean	0,476	0,019	32,7 %	32,7 %
	SD	0,086	0,002	3,0 %	3,7 %
3h	B8-1	1,165	0,050	88,1 %	
	B8-2	1,145	0,050	86,5 %	87,3 %
	B9-1	1,095	0,046	80,3 %	
	B9-2	1,090	0,046	80,0 %	80,1 %
	mean	1,124	0,048	83,7 %	83,7 %
	SD	0,037	0,002	4,2 %	5,1 %
24h	B18-1	1,213	0,063	110,3 %	
	B18-2	1,175	0,061	106,8 %	108,6 %
	B19-1	1,050	0,053	92,1 %	
	B19-2	1,034	0,052	90,7 %	91,4 %
	mean	1,118	0,057	100,0 %	100,0 %
	SD	0,089	0,006	10,0 %	12,1 %

EGN-02-01: Effects of exposure time of menadione on rat liver slices

Groups: Group A (menadione 200 uM / DMSO 1%)
Group B (DMSO 1%) B1-B5 (0h-control) - not treated

background: medium		1	0	0,2	41
Culture media enzyme activities (U/L); raw data					
time	sample	ASAT	ALAT	GLDH	LDH
3h	A6	40	19	2,5	587
	A7	35	16	2,2	558
	A8	43	20	3,3	673
	A9	37	24	2,6	614
	A10	41	23	2,1	698
	mean	39,2	20,4	2,5	626
	SD	3,2	3,2	0,5	58
6h	A11	86	31	7,8	1 123
	A12	96	34	10,3	1 287
	A13	118	38	12,0	1 522
	A14	85	30	7,0	1 170
	A15	116	34	14,6	1 238
	mean	100,2	33,4	10,3	1 268
	SD	15,9	3,1	3,1	155
24h	A16	119	14	16,4	1 739
	A17	117	12	18,0	1 474
	A18	131	16	17,6	1 791
	A19	138	14	25,2	1 708
	A20	129	19	17,2	1 826
	mean	126,8	15,0	18,9	1 708
	SD	8,7	2,6	3,6	138
0h					
3h	B6	31	16	2,2	454
	B7	28	14	2,2	392
	B8	28	16	1,7	440
	B9	30	16	2,6	403
	B10	34	19	3,5	444
	mean	30,2	16,2	2,4	427
	SD	2,5	1,8	0,7	27
6h	B11	47	21	7,7	552
	B12	71	23	14,3	606
	B13	63	21	11,5	552
	B14	72	24	14,2	670
	B15	89	24	18,4	734
	mean	68,4	22,6	13,2	623
	SD	15,3	1,5	3,9	79
24h	B16	140	25	32,7	1 090
	B17	88	18	24,8	664
	B18	104	20	31,1	780
	B19	119	21	40,1	907
	B20	159	27	47,3	1 207
	mean	122,0	22,2	35,2	930
	SD	28,2	3,7	8,7	221

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
3h	A6				
	A7				
	A8				
	A9				
	A10	159	23	95,4	448
	mean	159,0	23,0	95,4	448
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	A11				
	A12				
	A13	109	9	72,9	156
	A14	137	15	85,0	229
	A15	132	15	85,2	254
	mean	126,0	13,0	81,0	213
	SD	14,9	3,5	7,0	51
24h	A16				
	A17				
	A18				
	A19				
	A20	126	8	66,5	119
	mean	126,0	8,0	66,5	119
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
0h	B1				
	B2				
	B3	100	25	86,3	496
	B4	111	29	89,7	543
	B5	115	25	93,4	473
	mean	108,7	26,3	89,8	504
	SD	7,8	2,3	3,6	36
3h	B6				
	B7				
	B8				
	B9				
	B10	117	27	90,9	424
	mean	117,0	27,0	90,9	424
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	B11				
	B12				
	B13	121	22	89,6	349
	B14	130	24	94,0	374
	B15	142	22	98,6	389
	mean	131,0	22,7	94,1	371
	SD	10,5	1,2	4,5	20
24h	B16				
	B17				
	B18				
	B19				
	B20	116	9	74,6	177
	mean	116,0	9,0	74,6	177
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-03-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: Group A (untreated / only pre-incubation)
Group B (negative control)
Group C (ferrous sulphate 100 uM)

Slice weights (mg)

time	sample	weight	comments
0h	A2	23,6	
	A3	45,6	looked very large and thick; two slices stick tohether?
	A4	22,5	
	A5	23,3	
	mean	28,8	
	SD	11,2	
3h	B1	46,2	looked very large and thick; two slices stick tohether?
	B2	21,4	
	B3	21,5	
	B4	44,4	looked very large and thick; two slices stick tohether?
	B5	42,3	looked very large and thick; two slices stick tohether?
	B6	20,9	
	mean	32,8	
	SD	12,7	
6h	B7	31,0	
	B8	27,9	
	B9	FALSE	slice missing after incubation
	B10	23,1	
	mean	27,3	
	SD	4,0	
24h	B11	21,2	
	B12	20,9	
	B13	16,1	
	B14	16,4	
	B15	16,2	
	B16	16,8	
	mean	17,9	
	SD	2,4	
3h	C1	22,0	
	C2	20,7	
	C3	39,0	looked very large and thick; two slices stick tohether?
	C4	20,4	
	C5	44,3	looked very large and thick; two slices stick tohether?
	C6	20,1	
	C7	17,5	
	mean	26,3	
	SD	10,7	
6h	C8	21,5	
	C9	24,5	
	C10	28,8	
	C11	27,4	
	C12	21,4	
	mean	24,7	
	SD	3,4	
24h	C13	22,9	
	C14	23,2	
	C15	19,1	
	C16	21,1	
	C17	18,7	
	C18	19,0	
	C19	19,9	
	mean	20,6	
	SD	1,9	

EGN-03-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: Group A (untreated / only pre-incubation)
Group B (negative control)
Group C (ferrous sulphate 100 uM)

MTT test (OD-570); raw data minus background					**Abs.570nm / mg slice wet weight**
time	sample	OD-570	versus weight	and v. 24-h neg. (%)	slice
3h	B5-1	0,921	0,022	31,6 %	
	B5-2	0,901	0,021	30,9 %	31,3 %
	B6-1	1,203	0,058	83,6 %	
	B6-2	1,166	0,056	81,0 %	82,3 %
	mean	1,048	0,039	56,8 %	56,8 %
	SD	0,159	0,020	29,5 %	36,1 %
24h	B15-1	1,016	0,063	91,1 %	
	B15-2	0,997	0,062	89,4 %	90,2 %
	B16-1	1,295	0,077	111,9 %	
	B16-2	1,246	0,074	107,7 %	109,8 %
	mean	1,139	0,069	100,0 %	100,0 %
	SD	0,154	0,008	11,5 %	13,9 %
3h	C6-1	1,036	0,052	74,8 %	
	C6-2	1,026	0,051	74,1 %	74,5 %
	C7-1	1,307	0,075	108,4 %	
	C7-2	1,274	0,073	105,7 %	107,1 %
	mean	1,161	0,063	90,8 %	90,8 %
	SD	0,150	0,013	18,9 %	23,0 %
24h	C18-1	0,640	0,034	48,9 %	
	C18-2	0,626	0,033	47,8 %	48,4 %
	C19-1	1,040	0,052	75,9 %	
	C19-2	1,012	0,051	73,8 %	74,9 %
	mean	0,830	0,042	61,6 %	61,6 %
	SD	0,227	0,011	15,3 %	18,7 %

EGN-03-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: Group A (untreated / only pre-incubation)
Group B (negative control)
Group C (ferrous sulphate 100 uM)

background: medium		3	1	0,4	35
Culture media enzyme activities (U/L); raw data					
time	sample	ASAT	ALAT	GLDH	LDH
0h					
3h	B1	58	37	2,2	1 065
	B2	19	11	1,6	401
	B3	26	14	2,0	442
	B4	69	43	2,6	1 296
	B5	53	32	2,4	990
	B6	27	14	1,6	482
	mean	42,0	25,2	2,1	779
	SD	20,6	13,8	0,4	384
6h	B7	86	40	10,9	1 182
	B8	96	38	15,1	1 193
	B9	FALSE	FALSE	FALSE	FALSE
	B10	60	24	11,0	843
	mean	80,7	34,0	12,3	1 073
	SD	18,6	8,7	2,4	199
24h	B11	122	27	29,2	1 194
	B12	114	25	31,1	1 065
	B13	59	14	14,5	552
	B14	56	15	14,1	543
	B15	84	19	18,8	747
	B16	60	13	15,0	540
	mean	82,5	18,8	20,5	774
	SD	29,4	5,9	7,7	290
3h	C1	25	14	2,2	426
	C2	17	9	1,7	324
	C3	62	39	4,2	1 168
	C4	21	9	1,4	405
	C5	56	35	2,0	1 077
	C6	21	11	1,4	444
	C7	15	7	1,1	258
	mean	31,0	17,7	2,0	586
	SD	19,5	13,4	1,0	373
6h	C8	72	28	12,4	989
	C9	63	25	11,8	842
	C10	70	33	9,7	1 114
	C11	68	26	11,8	979
	C12	58	22	8,8	807
	mean	66,2	26,8	10,9	946
	SD	5,7	4,1	1,6	124
24h	C13	179	30	38,0	1 913
	C14	138	23	30,8	1 555
	C15	107	25	24,1	1 034
	C16	138	22	40,5	1 420
	C17	118	23	28,1	1 158
	C18	150	26	40,1	1 329
	C19	103	22	27,4	1 050
	mean	133,3	24,4	32,7	1 351
	SD	26,6	2,9	6,7	314

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h	A2				
	A3				
	A4				
	A5	67	21	63,8	417
	mean	67,0	21,0	63,8	417
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3h	B1				
	B2				
	B3				
	B4	181	23	99,3	462
	B5				
	B6				
	mean	181,0	23,0	99,3	462
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	B7				
	B8				
	B9				
	B10	87	18	75,6	350
	mean	87,0	18,0	75,6	350
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B11				
	B12				
	B13				
	B14	109	12	56,3	254
	B15				
	B16				
	mean	109,0	12,0	56,3	254
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3h	C1				
	C2				
	C3				
	C4				
	C5	202	24	100,5	486
	C6				
	C7				
	mean	202,0	24,0	100,5	486
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	C8				
	C9				
	C10				
	C11				
	C12	92	17	72,6	332
	mean	92,0	17,0	72,6	332
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C13				
	C14				
	C15				
	C16				
	C17	98	10	60,8	193
	C18				
	C19				
	mean	98,0	10,0	60,8	193
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-05-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: Group A (untreated / only pre-incubation)
Group B (negative control)
Group C (ferrous sulphate 100 uM)

Slice weights (mg)

time	sample	weight	comments
0h	A2	21,5	
	A3	24,8	Regarding all slices:
	A4	20,8	Slices were prepared from two livers instead of one,
	A5	25,6	and were pooled to some degree before they were
	mean	23,2	divided into treatment groups and incubated.
	SD	2,4	
3h	B1	24,2	
	B2	24,4	
	B3	23,5	
	B4	26,1	
	B5	23,0	
	B6	22,8	
	mean	24,0	
	SD	1,2	
6h	B7	26,8	
	B8	21,1	
	B9	22,0	
	B10	25,2	
	mean	23,8	
	SD	2,7	
24h	B11	17,2	
	B12	18,5	
	B13	18,0	
	B14	19,2	
	B15	19,7	
	B16	18,3	
	mean	18,5	
	SD	0,9	
3h	C1	26,4	
	C2	19,4	
	C3	26,0	
	C4	22,7	
	C5	23,0	
	C6	24,9	
	C7	20,9	
	mean	23,3	
	SD	2,6	
6h	C8	35,5	
	C9	23,8	
	C10	21,7	
	C11	27,2	
	C12	25,3	
	mean	26,7	
	SD	5,3	
24h	C13	17,4	
	C14	16,0	
	C15	18,7	
	C16	19,3	
	C17	16,7	
	C18	18,5	
	C19	21,4	
	mean	18,3	
	SD	1,8	

EGN-05-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: Group A (untreated / only pre-incubation)
Group B (negative control)
Group C (ferrous sulphate 100 uM)

MTT test (OD-570); raw data minus background

time	sample	OD-570	versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight** slice
3h	B5-1	1,410	0,061	91,4 %	
	B5-2	1,398	0,061	90,7 %	91,1 %
	B6-1	1,297	0,057	84,9 %	
	B6-2	1,274	0,056	83,4 %	84,1 %
	mean	1,345	0,059	87,6 %	87,6 %
	SD	0,069	0,003	4,1 %	4,9 %
24h	B15-1	1,239	0,063	93,8 %	
	B15-2	1,222	0,062	92,5 %	93,2 %
	B16-1	1,324	0,072	107,9 %	
	B16-2	1,297	0,071	105,7 %	106,8 %
	mean	1,271	0,067	100,0 %	100,0 %
	SD	0,048	0,005	7,9 %	9,7 %
3h	C6-1	1,525	0,061	91,4 %	
	C6-2	1,488	0,060	89,1 %	90,3 %
	C7-1	1,472	0,070	105,1 %	
	C7-2	1,470	0,070	104,9 %	105,0 %
	mean	1,489	0,065	97,6 %	97,6 %
	SD	0,025	0,006	8,6 %	10,4 %
24h	C18-1	0,887	0,048	71,5 %	
	C18-2	0,878	0,047	70,8 %	71,2 %
	C19-1	1,017	0,048	70,9 %	
	C19-2	0,989	0,046	68,9 %	69,9 %
	mean	0,943	0,047	70,5 %	70,5 %
	SD	0,071	0,001	1,1 %	0,9 %

EGN-05-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: Group A (untreated / only pre-incubation)
Group B (negative control)
Group C (ferrous sulphate 100 uM)

background: medium 4 0 59

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
3h	B1	36	13	2,7	465
	B2	39	16	2,5	526
	B3	45	16	2,2	566
	B4	48	20	3,1	646
	B5	25	13	1,9	403
	B6	35	25	1,8	480
	mean	38,0	17,2	2,4	514
	SD	8,1	4,6	0,5	85
6h	B7	47	23	5,0	647
	B8	47	24	6,0	571
	B9	48	25	7,1	572
	B10	59	37	5,6	842
	mean	50,3	27,3	5,9	658
	SD	5,9	6,6	0,9	128
24h	B11	98	37	20,5	793
	B12	99	36	25,0	821
	B13	87	32	22,7	676
	B14	71	27	14,4	535
	B15	81	28	16,3	669
	B16	60	21	14,2	488
	mean	82,7	30,2	18,9	664
	SD	15,3	6,0	4,5	133
3h	C1	37	16	2,5	513
	C2	24	9	1,6	316
	C3	40	28	2,0	545
	C4	35	15	1,6	512
	C5	41	17	2,0	559
	C6	37	13	2,1	524
	C7	36	14	2,1	464
	mean	35,7	16,0	2,0	490
	SD	5,6	5,9	0,3	83
6h	C8	80	47	13,3	949
	C9	47	24	7,2	550
	C10	54	26	8,7	558
	C11	60	36	6,4	787
	C12	66	36	11,3	703
	mean	61,4	33,8	9,4	709
	SD	12,6	9,2	2,9	167
24h	C13	119	47	25,7	1 003
	C14	78	30	19,6	605
	C15	92	35	21,9	785
	C16	68	27	15,1	584
	C17	139	60	26,5	1 171
	C18	190	75	37,6	1 689
	C19	122	44	21,8	1 059
	mean	115,4	45,4	24,0	985
	SD	41,6	17,2	7,1	383

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h	A2				
	A3				
	A4				
	A5	102	44	76,3	564
	mean	102,0	44,0	76,3	564
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3h	B1				
	B2				
	B3				
	B4	100	39	75,4	399
	B5				
	B6				
	mean	100,0	39,0	75,4	399
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	B7				
	B8				
	B9				
	B10	111	45	77,3	312
	mean	111,0	45,0	77,3	312
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B11				
	B12				
	B13				
	B14	125	46	74,9	299
	B15				
	B16				
	mean	125,0	46,0	74,9	299
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
3h	C1				
	C2				
	C3				
	C4				
	C5	104	40	64,1	348
	C6				
	C7				
	mean	104,0	40,0	64,1	348
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
6h	C8				
	C9				
	C10				
	C11				
	C12	100	46	74,2	322
	mean	100,0	46,0	74,2	322
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C13				
	C14				
	C15				
	C16				
	C17	155	48	82,5	271
	C18				
	C19				
	mean	155,0	48,0	82,5	271
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-06-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 100 uM)
E (24h FeSO4 200 uM)
F (24h FeSO4 400 uM)

Slice weights (n=6 or 7) **mg**

Treatment category (untreated, ne			Analysis category (histology n=9, triton n=6, iron n=12, MTT n=12)		
	sample	weight		sample	weight
A			histology		
	A2	15,1		A2	15,1
	A3	19,3		B2	16,9
	A4	17,1		C2	20,0
	A5	21,7		D1	19,3
	A6	17,6		D2	16,5
	A7	16,6		E1	17,9
	mean	17,9		E2	19,5
	SD	2,3		F1	19,8
B	B2	16,9		F2	19,3
	B3	16,6		mean	18,3
	B4	18,7		SD	1,7
	B5	14,1	triton	A5	21,7
	B6	15,7		B5	14,1
	B7	14,9		C5	22,9
	mean	16,2		D5	14,2
	SD	1,6		E5	16,3
C	C2	20,0		F5	15,2
	C3	20,1		mean	17,4
	C4	20,3		SD	3,9
	C5	22,9	iron	A3	19,3
	C6	20,3		A4	17,1
	C7	17,9		B3	16,6
	mean	20,3		B4	18,7
	SD	1,6		C3	20,1
D	D1	19,3		C4	20,3
	D2	16,5		D3	19,9
	D3	19,9		D4	13,3
	D4	13,3		E3	16,4
	D5	14,2		E4	19,5
	D6	14,8		F3	16,1
	D7	18,6		F4	15,6
	mean	16,7		mean	17,7
	SD	2,6		SD	2,2
E	E1	17,9	MTT	A6	17,6
	E2	19,5		A7	16,6
	E3	16,4		B6	15,7
	E4	19,5		B7	14,9
	E5	16,3		C6	20,3
	E6	16,2		C7	17,9
	E7	15,7		D6	14,8
	mean	17,4		D7	18,6
	SD	1,6		E6	16,2
F	F1	19,8		E7	15,7
	F2	19,3		F6	17,1
	F3	16,1		F7	15,8
	F4	15,6		mean	16,8
	F5	15,2		SD	1,6
	F6	17,1			
	F7	15,8			
	mean	17,0			
	SD	1,9			

EGN-06-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 100 uM)
E (24h FeSO4 200 uM)
F (24h FeSO4 400 uM)

Comment: The isopropanol extract of slice B6 had partly evaporated (about 1/3 was missing), and the MTT-value was too high. The cause of the evaporation has not been established.

MTT test (n=2; each with 2 parallels) (B: n=1; with 2 parallels)

time	sample	OD-570		versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight**
0h	A6-1	1,210		0,069	93,6 %	
	A6-2	1,175		0,067	90,9 %	92,2 %
	A7-1	1,321		0,080	108,3 %	
	A7-2	1,297		0,078	106,4 %	107,4 %
	mean	1,264		0,073	99,8 %	99,8 %
	SD	0,078		0,006	8,8 %	10,7 %
24h	B6-1	FALSE	1,783	FALSE	FALSE	
	B6-2	FALSE	1,724	FALSE	FALSE	FALSE
	B7-1	1,090		0,073	99,6 %	
	B7-2	1,099		0,074	100,4 %	100,0 %
	mean	1,095		0,073	100,0 %	100,0 %
	SD	0,006		0,000	0,6 %	#DIV/0!
24h	C6-1	0,343		0,017	23,0 %	
	C6-2	0,338		0,017	22,7 %	22,8 %
	C7-1	0,410		0,023	31,2 %	
	C7-2	0,405		0,023	30,8 %	31,0 %
	mean	0,384		0,020	26,9 %	26,9 %
	SD	0,040		0,003	4,7 %	5,8 %
24h	D6-1	1,200		0,081	110,4 %	
	D6-2	1,166		0,079	107,3 %	108,8 %
	D7-1	1,096		0,059	80,2 %	
	D7-2	1,086		0,058	79,5 %	79,9 %
	mean	1,116		0,069	94,3 %	94,3 %
	SD	0,044		0,012	16,8 %	20,5 %
24h	E6-1	1,053		0,065	88,5 %	
	E6-2	1,052		0,065	88,4 %	88,4 %
	E7-1	1,480		0,094	128,3 %	
	E7-2	1,448		0,092	125,6 %	126,9 %
	mean	1,258		0,079	107,7 %	107,7 %
	SD	0,238		0,016	22,3 %	27,2 %
24h	F6-1	1,296		0,076	103,2 %	
	F6-2	1,294		0,076	103,0 %	103,1 %
	F7-1	1,296		0,082	111,7 %	
	F7-2	1,294		0,082	111,5 %	111,6 %
	mean	1,295		0,079	107,3 %	107,3 %
	SD	0,001		0,004	4,9 %	6,0 %

EGN-06-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups:		Titles:	
	A (untreated / pre-inc.)		Culture media
	B (24h neg.ctrl.)		activities
	C (24h pos.ctrl.; menadione 200 uM)		leakage
	D (24h FeSO4 100 uM)		(n=4, 5, 6, or 7)
	E (24h FeSO4 200 uM)		U/L
	F (24h FeSO4 400 uM)		% of total

background: medium		3	0	0,2	43

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
24h	B2	66	16	11,8	513
	B3	58	14	11,9	408
	B4	108	22	26,1	668
	B5	38	9	5,2	316
	B6	70	12	15,3	463
	B7	70	14	17,2	517
	mean	68,3	14,5	14,6	480,8
	SD	22,9	4,4	7,0	118,5
24h	C2	94	11	14,1	1 255
	C3	106	17	11,4	1 349
	C4	118	17	12,8	1 293
	C5	100	16	12,4	1 443
	C6	94	10	8,4	1 304
	C7	101	12	10,0	1 173
	mean	102,2	13,8	11,5	1 302,8
	SD	9,0	3,2	2,1	90,6
24h	D1	63	12	12,0	400
	D2	93	18	18,3	657
	D3	143	26	36,9	897
	D4	79	18	21,5	595
	D5	74	16	15,6	494
	D6	88	22	15,9	579
	D7	113	21	25,0	803
	mean	93,3	19,0	20,7	632,1
	SD	27,0	4,5	8,3	171,8
24h	E1	106	16	27,0	543
	E2	113	20	32,5	647
	E3	127	24	30,3	695
	E4	125	25	27,0	814
	E5	65	12	13,7	365
	E6	105	17	26,7	629
	E7	66	12	13,5	311
	mean	101,0	18,0	24,4	572,0
	SD	25,7	5,3	7,7	180,0
24h	F1	140	25	32,3	895
	F2	72	15	15,1	388
	F3	100	23	20,6	604
	F4	87	20	17,6	513
	F5	103	20	27,3	672
	F6	101	19	24,4	533
	F7	64	12	15,0	359
	mean	95,3	19,1	21,8	566,3
	SD	24,8	4,5	6,6	182,3

Culture media ASAT activities (n=4, 5, 6, or 7)	ASAT leakage (n=4, 5, 6, or 7)	% of total ASAT
Culture media ALAT activities (n=4, 5, 6, or 7)	ALAT leakage (n=4, 5, 6, or 7)	% of total ALAT
Culture media GLDH activities (n=4, 5, 6, or 7	GLDH leakage (n=4, 5, 6, or 7)	% of total GLDH
Culture media LDH activities (n=4, 5, 6, or 7)	LDH leakage (n=4, 5, 6, or 7)	% of total LDH

Comment: **Noise occurred while measuring triton values of slice E5, thus omitted**.
Triton supernatant enzyme activities (n=1) (E: n=0; values are calculated as averages of D and F)

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
	A2				
	A3				
	A4				
	A5	91	29	66,1	387
	A6				
	A7				
	mean	91,0	29,0	66,1	387,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B2				
	B3				
	B4				
	B5	108	15	34,8	156
	B6				
	B7				
	mean	108,0	15,0	34,8	156,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C2				
	C3				
	C4				
	C5	81	9	37,0	98
	C6				
	C7				
	mean	81,0	9,0	37,0	98,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	D1				
	D2				
	D3				
	D4				
	D5	95	14	26,1	151
	D6				
	D7				
	mean	95,0	14,0	26,1	151,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	E1				
	E2				
	E3				
	E4				
	E5	116	16	57,1	261
	E6				
	E7				
	mean	91,5	12,5	20,1	153,5
	SD	4,9	2,1	8,5	3,5
24h	F1				
	F2				
	F3				
	F4				
	F5	88	11	14,1	156
	F6				
	F7				
	mean	88,0	11,0	14,1	156,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-07-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 100 uM)
E (24h FeSO4 200 uM)
F (24h FeSO4 400 uM)

Slice weights (n=6 or 7) **mg**

Treatment category (untreated, ne	sample	weight	Analysis category (histology n=9, triton n=6, iron n=12, MTT n=12)	sample	weight
A			histology		
	A2	29,9		A2	29,9
	A3	27,0		B2	23,9
	A4	26,4		C2	26,2
	A5	28,6		D1	24,5
	A6	28,4		D2	21,6
	A7	29,6		E1	26,5
	mean	28,3		E2	23,1
	SD	1,4		F1	26,8
B	B2	23,9		F2	25,8
	B3	24,1		mean	25,4
	B4	21,5		SD	2,4
	B5	23,6	triton	A5	28,6
	B6	23,1		B5	23,6
	B7	18,8		C5	29,4
	mean	22,5		D5	24,9
	SD	2,0		E5	28,6
C	C2	26,2		F5	25,1
	C3	33,6		mean	26,7
	C4	32,8		SD	2,4
	C5	29,4	iron	A3	27,0
	C6	29,7		A4	26,4
	C7	24,7		B3	24,1
	mean	29,4		B4	21,5
	SD	3,5		C3	33,6
D	D1	24,5		C4	32,8
	D2	21,6		D3	23,5
	D3	23,5		D4	23,9
	D4	23,9		E3	20,7
	D5	24,9		E4	16,2
	D6	22,6		F3	21,9
	D7	19,4		F4	23,1
	mean	22,9		mean	24,6
	SD	1,9		SD	4,9
E	E1	26,5	MTT	A6	28,4
	E2	23,1		A7	29,6
	E3	20,7		B6	23,1
	E4	16,2		B7	18,8
	E5	28,6		C6	29,7
	E6	23,5		C7	24,7
	E7	23,7		D6	22,6
	mean	23,2		D7	19,4
	SD	4,0		E6	23,5
F	F1	26,8		E7	23,7
	F2	25,8		F6	22,2
	F3	21,9		F7	22,3
	F4	23,1		mean	24,0
	F5	25,1		SD	3,6
	F6	22,2			
	F7	22,3			
	mean	23,9			
	SD	2,0			

EGN-07-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 100 uM)
E (24h FeSO4 200 uM)
F (24h FeSO4 400 uM)

MTT test (n=2; each with 2 parallels)

time	sample	OD-570	versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight** slice
0h	A6-1	1,011	0,036	115,6 %	
	A6-2	0,968	0,034	110,7 %	113,1 %
	A7-1	0,856	0,029	93,9 %	
	A7-2	0,859	0,029	94,2 %	94,1 %
	mean	0,894	0,032	103,6 %	103,6 %
	SD	0,064	0,003	11,2 %	13,5 %
24h	B6-1	0,786	0,034	110,5 %	
	B6-2	0,779	0,034	109,5 %	110,0 %
	B7-1	0,519	0,028	89,6 %	
	B7-2	0,523	0,028	90,3 %	90,0 %
	mean	0,607	0,031	100,0 %	100,0 %
	SD	0,149	0,004	11,6 %	14,1 %
24h	C6-1	0,457	0,015	50,0 %	
	C6-2	0,442	0,015	48,3 %	49,1 %
	C7-1	0,313	0,013	41,2 %	
	C7-2	0,312	0,013	41,0 %	41,1 %
	mean	0,356	0,014	45,1 %	45,1 %
	SD	0,075	0,001	4,7 %	5,7 %
24h	D6-1	0,885	0,039	127,2 %	
	D6-2	0,853	0,038	122,6 %	124,9 %
	D7-1	0,568	0,029	95,1 %	
	D7-2	0,555	0,029	92,9 %	94,0 %
	mean	0,659	0,034	109,4 %	109,4 %
	SD	0,168	0,006	17,9 %	21,8 %
24h	E6-1	0,916	0,039	126,6 %	
	E6-2	0,915	0,039	126,4 %	126,5 %
	E7-1	0,520	0,022	71,3 %	
	E7-2	0,504	0,021	69,1 %	70,2 %
	mean	0,714	0,030	98,3 %	98,3 %
	SD	0,233	0,010	32,5 %	39,8 %
24h	F6-1	0,537	0,024	78,6 %	
	F6-2	0,526	0,024	76,9 %	77,7 %
	F7-1	0,796	0,036	115,9 %	
	F7-2	0,767	0,034	111,7 %	113,8 %
	mean	0,657	0,029	95,8 %	95,8 %
	SD	0,145	0,006	20,9 %	25,5 %

EGN-07-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups:	A (untreated / pre-inc.)	Titles:	Culture media
	B (24h neg.ctrl.)		activities
	C (24h pos.ctrl.; menadione 200 uM)		leakage
	D (24h FeSO4 100 uM)		(n=4, 5, 6, or 7)
	E (24h FeSO4 200 uM)		U/L
	F (24h FeSO4 400 uM)		% of total

background: medium	0	3	0,1	46

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
24h	B2	130	22	37,7	1 368
	B3	131	28	26,4	1 536
	B4	91	8	19,6	1 012
	B5	137	29	32,7	1 093
	B6	97	19	21,5	1 027
	B7	63	14	12,5	550
	mean	108,2	20,0	25,1	1 097,7
	SD	29,3	8,1	9,2	339,9
24h	C2	102	16	12,5	1 753
	C3	111	14	17,8	2 156
	C4	127	16	23,1	2 071
	C5	106	16	16,7	2 238
	C6	106	21	13,6	1 741
	C7	98	11	14,1	1 704
	mean	108,3	15,7	16,3	1 943,8
	SD	10,1	3,3	3,9	237,8
24h	D1	111	29	25,7	1 300
	D2	121	26	31,3	1 236
	D3	126	22	33,6	1 220
	D4	122	27	22,1	1 172
	D5	139	34	33,5	1 591
	D6	111	22	27,5	1 127
	D7	78	22	17,1	824
	mean	115,4	26,0	27,3	1 210,0
	SD	19,1	4,5	6,2	227,8
24h	E1	139	34	32,2	1 495
	E2	153	38	35,1	1 354
	E3	98	27	22,5	1 067
	E4	95	20	18,9	910
	E5	158	28	39,8	1 944
	E6	155	28	39,8	1 392
	E7	131	25	30,7	1 499
	mean	132,7	28,6	31,3	1 380,1
	SD	26,5	5,9	8,1	333,0
24h	F1	138	32	32,6	1 708
	F2	170	32	44,2	1 486
	F3	119	28	25,4	1 145
	F4	141	32	33,2	1 494
	F5	155	31	36,8	1 503
	F6	139	29	31,4	1 462
	F7	137	29	29,0	1 157
	mean	142,7	30,4	33,2	1 422,1
	SD	16,0	1,7	6,0	202,6

Culture media ASAT activities (n=4, 5, 6, or 7) ASAT leakage (n=4, 5, 6, or 7) % of total ASAT
Culture media ALAT activities (n=4, 5, 6, or 7) ALAT leakage (n=4, 5, 6, or 7) % of total ALAT
Culture media GLDH activities (n=4, 5, 6, or 7 GLDH leakage (n=4, 5, 6, or 7) % of total GLDH
Culture media LDH activities (n=4, 5, 6, or 7) LDH leakage (n=4, 5, 6, or 7) % of total LDH

Triton supernatant enzyme activities (n=1)

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
	A2				
	A3				
	A4				
	A5	100	32	74,5	604
	A6				
	A7				
	mean	100,0	32,0	74,5	604,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B2				
	B3				
	B4				
	B5	111	13	63,2	273
	B6				
	B7				
	mean	111,0	13,0	63,2	273,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C2				
	C3				
	C4				
	C5	99	7	41,9	106
	C6				
	C7				
	mean	99,0	7,0	41,9	106,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	D1				
	D2				
	D3				
	D4				
	D5	109	10	52,9	182
	D6				
	D7				
	mean	109,0	10,0	52,9	182,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	E1				
	E2				
	E3				
	E4				
	E5	93	6	42,7	111
	E6				
	E7				
	mean	93,0	6,0	42,7	111,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	F1				
	F2				
	F3				
	F4				
	F5	102	10	49,5	158
	F6				
	F7				
	mean	102,0	10,0	49,5	158,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-09-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

Comments: A7 had a small and uneven diameter, which may explain the low weight and possibly other deviant parameters.

Slice weights (n=6 or 7) **mg**

Treatment category (untreated, ne	sample	weight	Analysis category (histology n=9, triton n=6, iron n=12, MTT n=12)	sample	weight
A			histology		
	A2	32,2		A2	32,2
	A3	29,9		B2	25,3
	A4	33,4		C2	26,5
	A5	32,9		D1	23,0
	A6	28,5		D2	25,5
	A7	22,2		E1	33,6
	mean	29,9		E2	25,0
	SD	4,2		F1	24,1
B	B2	25,3		F2	30,7
	B3	25,1		mean	27,3
	B4	24,3		SD	3,8
	B5	24,8	triton	A5	32,9
	B6	21,7		B5	24,8
	B7	23,9		C5	28,9
	mean	24,2		D5	24,4
	SD	1,3		E5	26,5
C	C2	26,5		F5	25,7
	C3	24,7		mean	27,2
	C4	28,8		SD	3,2
	C5	28,9	iron	A3	29,9
	C6	27,3		A4	33,4
	C7	23,2		B3	25,1
	mean	26,6		B4	24,3
	SD	2,3		C3	24,7
D	D1	23,0		C4	28,8
	D2	25,5		D3	20,4
	D3	20,4		D4	24,8
	D4	24,8		E3	28,0
	D5	24,4		E4	29,5
	D6	17,9		F3	25,3
	D7	29,1		F4	25,9
	mean	23,6		mean	26,7
	SD	3,6		SD	3,4
E	E1	33,6	MTT	A6	28,5
	E2	25,0		A7	22,2
	E3	28,0		B6	21,7
	E4	29,5		B7	23,9
	E5	26,5		C6	27,3
	E6	26,9		C7	23,2
	E7	23,3		D6	17,9
	mean	27,5		D7	29,1
	SD	3,3		E6	26,9
F	F1	24,1		E7	23,3
	F2	30,7		F6	26,1
	F3	25,3		F7	29,5
	F4	25,9		mean	25,0
	F5	25,7		SD	3,5
	F6	26,1			
	F7	29,5			
	mean	26,8			
	SD	2,4			

EGN-09-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

MTT test (n=2; each with 2 parallels)

time	sample	OD-570	versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight** slice
0h	A6-1	1,434	0,050	165,9 %	
	A6-2	1,394	0,049	161,3 %	163,6 %
	A7-1	0,683	0,031	101,5 %	
	A7-2	0,674	0,030	100,1 %	100,8 %
	mean	0,917	0,040	132,2 %	132,2 %
	SD	0,413	0,011	36,3 %	44,4 %
24h	B6-1	0,727	0,034	110,5 %	
	B6-2	0,704	0,032	107,0 %	108,7 %
	B7-1	0,673	0,028	92,9 %	
	B7-2	0,650	0,027	89,7 %	91,3 %
	mean	0,676	0,030	100,0 %	100,0 %
	SD	0,027	0,003	10,3 %	12,3 %
24h	C6-1	0,243	0,009	29,4 %	
	C6-2	0,232	0,008	28,0 %	28,7 %
	C7-1	0,228	0,010	32,4 %	
	C7-2	0,219	0,009	31,1 %	31,8 %
	mean	0,226	0,009	30,2 %	30,2 %
	SD	0,007	0,001	1,9 %	2,2 %
24h	D6-1	0,744	0,042	137,1 %	
	D6-2	0,753	0,042	138,7 %	137,9 %
	D7-1	0,236	0,008	26,7 %	
	D7-2	0,230	0,008	26,1 %	26,4 %
	mean	0,406	0,025	82,1 %	82,1 %
	SD	0,300	0,020	64,4 %	78,8 %
24h	E6-1	0,448	0,017	54,9 %	
	E6-2	0,442	0,016	54,2 %	54,6 %
	E7-1	0,701	0,030	99,2 %	
	E7-2	0,695	0,030	98,4 %	98,8 %
	mean	0,572	0,023	76,7 %	76,7 %
	SD	0,146	0,008	25,5 %	31,3 %
24h	F6-1	0,346	0,013	43,7 %	
	F6-2	0,343	0,013	43,3 %	43,5 %
	F7-1	0,381	0,013	42,6 %	
	F7-2	0,375	0,013	41,9 %	42,3 %
	mean	0,361	0,013	42,9 %	42,9 %
	SD	0,020	0,000	0,8 %	0,9 %

EGN-09-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

Titles: Culture media activities leakage (n=4, 5, 6, or 7) U/L % of total

background: medium		5	1	0,3	56

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
24h	B2	166	47	34,7	1 917
	B3	137	41	25,3	1 600
	B4	168	43	39,0	1 702
	B5	114	36	28,6	1 551
	B6	101	30	18,2	1 242
	B7	130	29	21,9	1 547
	mean	136,0	37,7	28,0	1 593,2
	SD	27,1	7,3	7,8	220,9
24h	C2	102	32	3,6	1 905
	C3	87	23	5,2	1 626
	C4	118	23	6,6	1 903
	C5	119	32	8,9	2 091
	C6	23	7	3,3	1 640
	C7	109	21	6,0	1 726
	mean	93,0	23,0	5,6	1 815,2
	SD	36,3	9,2	2,1	182,4
24h	D1	153	41	34,2	1 586
	D2	122	39	25,7	1 605
	D3	127	30	22,3	1 289
	D4	143	44	31,8	1 541
	D5	127	43	27,1	1 520
	D6	93	30	16,6	1 014
	D7	178	41	37,6	2 070
	mean	134,7	38,3	27,9	1 517,9
	SD	26,8	5,9	7,2	322,5
24h	E1	172	52	39,4	2 580
	E2	174	42	37,6	1 871
	E3	155	42	36,6	1 876
	E4	167	43	41,2	2 145
	E5	148	36	34,4	1 735
	E6	136	34	25,6	1 588
	E7	133	28	21,0	1 512
	mean	155,0	39,6	33,7	1 901,0
	SD	16,8	7,7	7,5	364,9
24h	F1	219	40	66,8	1 481
	F2	207	49	58,3	1 678
	F3	192	37	55,4	1 443
	F4	195	41	53,1	1 433
	F5	193	45	51,0	1 592
	F6	193	36	46,8	1 563
	F7	216	34	55,8	1 703
	mean	202,1	40,3	55,3	1 556,1
	SD	11,7	5,3	6,3	109,1

Culture media ASAT activities (n=4, 5, 6, or 7) ASAT leakage (n=4, 5, 6, or 7) % of total ASAT
Culture media ALAT activities (n=4, 5, 6, or 7) ALAT leakage (n=4, 5, 6, or 7) % of total ALAT
Culture media GLDH activities (n=4, 5, 6, or 7 GLDH leakage (n=4, 5, 6, or 7) % of total GLDH
Culture media LDH activities (n=4, 5, 6, or 7) LDH leakage (n=4, 5, 6, or 7) % of total LDH

Triton supernatant enzyme activities (n=1)

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
	A2				
	A3				
	A4				
	A5	125	29	86,9	578
	A6				
	A7				
	mean	125,0	29,0	86,9	578,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B2				
	B3				
	B4				
	B5	105	7	41,6	345
	B6				
	B7				
	mean	105,0	7,0	41,6	345,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C2				
	C3				
	C4				
	C5	89	6	22,2	86
	C6				
	C7				
	mean	89,0	6,0	22,2	86,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	D1				
	D2				
	D3				
	D4				
	D5	104	7	34,6	176
	D6				
	D7				
	mean	104,0	7,0	34,6	176,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	E1				
	E2				
	E3				
	E4				
	E5	107	8	32,5	129
	E6				
	E7				
	mean	107,0	8,0	32,5	129,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	F1				
	F2				
	F3				
	F4				
	F5	84	7	23,6	93
	F6				
	F7				
	mean	84,0	7,0	23,6	93,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-10-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

Slice weights (n=6 or 7) **mg**

Treatment category (untreated, ne Analysis category (histology n=9, triton n=6, iron n=12, MTT n=12)

	sample	weight		sample	weight
A			histology		
	A2	21,7		A2	21,7
	A3	26,3		B2	16,6
	A4	28,7		C2	20,3
	A5	23,0		D1	17,5
	A6	28,1		D2	17,2
	A7	25,2		E1	19,2
	mean	25,5		E2	17,4
	SD	2,8		F1	22,6
B	B2	16,6		F2	14,5
	B3	17,5		mean	18,6
	B4	19,5		SD	2,6
	B5	15,3	triton	A5	23,0
	B6	16,6		B5	15,3
	B7	20,6		C5	20,3
	mean	17,7		D5	21,2
	SD	2,0		E5	16,8
C	C2	20,3		F5	20,0
	C3	22,2		mean	19,4
	C4	24,4		SD	2,9
	C5	20,3	iron	A3	26,3
	C6	20,5		A4	28,7
	C7	24,4		B3	17,5
	mean	22,0		B4	19,5
	SD	2,0		C3	22,2
D	D1	17,5		C4	24,4
	D2	17,2		D3	19,3
	D3	19,3		D4	20,8
	D4	20,8		E3	21,1
	D5	21,2		E4	18,2
	D6	20,1		F3	21,8
	D7	14,3		F4	18,7
	mean	18,6		mean	21,5
	SD	2,4		SD	3,4
E	E1	19,2	MTT	A6	28,1
	E2	17,4		A7	25,2
	E3	21,1		B6	16,6
	E4	18,2		B7	20,6
	E5	16,8		C6	20,5
	E6	20,4		C7	24,4
	E7	15,7		D6	20,1
	mean	18,4		D7	14,3
	SD	2,0		E6	20,4
F	F1	22,6		E7	15,7
	F2	14,5		F6	18,3
	F3	21,8		F7	21,0
	F4	18,7		mean	20,4
	F5	20,0		SD	4,0
	F6	18,3			
	F7	21,0			
	mean	19,6			
	SD	2,7			

EGN-10-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

MTT test (n=2; each with 2 parallels)

time	sample	OD-570	versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight** slice
0h	A6-1	0,964	0,034	66,4 %	
	A6-2	0,934	0,033	64,3 %	65,4 %
	A7-1	1,310	0,052	100,6 %	
	A7-2	1,285	0,051	98,7 %	99,6 %
	mean	1,176	0,043	82,5 %	82,5 %
	SD	0,210	0,010	19,8 %	24,2 %
24h	B6-1	0,793	0,048	92,4 %	
	B6-2	0,788	0,047	91,9 %	92,2 %
	B7-1	1,164	0,057	109,3 %	
	B7-2	1,132	0,055	106,3 %	107,8 %
	mean	1,028	0,052	100,0 %	100,0 %
	SD	0,208	0,005	9,1 %	11,1 %
24h	C6-1	0,604	0,029	57,0 %	
	C6-2	0,597	0,029	56,4 %	56,7 %
	C7-1	0,538	0,022	42,7 %	
	C7-2	0,526	0,022	41,7 %	42,2 %
	mean	0,554	0,026	49,4 %	49,4 %
	SD	0,038	0,004	8,4 %	10,2 %
24h	D6-1	0,788	0,039	75,9 %	
	D6-2	0,774	0,039	74,5 %	75,2 %
	D7-1	0,706	0,049	95,5 %	
	D7-2	0,691	0,048	93,5 %	94,5 %
	mean	0,724	0,044	84,9 %	84,9 %
	SD	0,044	0,006	11,2 %	13,7 %
24h	E6-1	0,868	0,043	82,3 %	
	E6-2	0,864	0,042	82,0 %	82,2 %
	E7-1	0,993	0,063	122,4 %	
	E7-2	0,961	0,061	118,5 %	120,4 %
	mean	0,922	0,052	101,3 %	101,3 %
	SD	0,065	0,011	22,2 %	27,1 %
24h	F6-1	0,852	0,047	90,1 %	
	F6-2	0,848	0,046	89,7 %	89,9 %
	F7-1	0,855	0,041	78,8 %	
	F7-2	0,840	0,040	77,4 %	78,1 %
	mean	0,849	0,043	84,0 %	84,0 %
	SD	0,007	0,004	6,8 %	8,3 %

EGN-10-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups:	A (untreated / pre-inc.)	Titles:	Culture media
	B (24h neg.ctrl.)		activities
	C (24h pos.ctrl.; menadione 200 uM)		leakage
	D (24h FeSO4 0.200 mM)		(n=4, 5, 6, or 7)
	E (24h FeSO4 1.00 mM)		U/L
	F (24h FeSO4 5.00 mM)		% of total

background: medium		2	1	0,1	49

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
24h	B2	83	11	18,1	671
	B3	94	14	21,4	759
	B4	102	15	16,1	1 075
	B5	82	11	18,9	655
	B6	139	13	40,7	934
	B7	132	14	23,8	1 145
	mean	105,3	13,0	23,2	873,2
	SD	24,6	1,7	9,0	209,7
24h	C2	104	10	12,1	1 357
	C3	104	9	12,8	1 422
	C4	116	10	15,9	1 624
	C5	103	10	12,5	1 302
	C6	93	9	9,6	1 372
	C7	120	11	12,2	1 572
	mean	106,7	9,8	12,5	1 441,5
	SD	9,8	0,8	2,0	128,2
24h	D1	115	12	22,1	918
	D2	159	19	33,6	1 257
	D3	218	18	62,9	1 345
	D4	148	16	30,6	1 345
	D5	133	16	30,0	1 129
	D6	104	13	22,7	967
	D7	78	11	14,5	682
	mean	136,4	15,0	30,9	1 091,9
	SD	45,2	3,1	15,5	248,5
24h	E1	117	16	25,0	861
	E2	115	15	25,8	927
	E3	169	19	38,9	1 146
	E4	127	17	28,8	1 078
	E5	136	15	35,3	993
	E6	147	18	32,5	1 083
	E7	79	11	17,5	622
	mean	127,1	15,9	29,1	958,6
	SD	28,3	2,6	7,2	177,9
24h	F1	259	26	70,1	1 452
	F2	129	14	32,2	792
	F3	246	26	57,7	1 413
	F4	231	23	63,1	1 254
	F5	158	20	37,7	1 058
	F6	239	17	67,0	1 174
	F7	193	19	49,5	1 072
	mean	207,9	20,7	53,9	1 173,6
	SD	49,2	4,5	14,6	227,5

Culture media ASAT activities (n=4, 5, 6, or 7) ASAT leakage (n=4, 5, 6, or 7) % of total ASAT
Culture media ALAT activities (n=4, 5, 6, or 7) ALAT leakage (n=4, 5, 6, or 7) % of total ALAT
Culture media GLDH activities (n=4, 5, 6, or 7 GLDH leakage (n=4, 5, 6, or 7) % of total GLDH
Culture media LDH activities (n=4, 5, 6, or 7) LDH leakage (n=4, 5, 6, or 7) % of total LDH

Triton supernatant enzyme activities (n=1)

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
	A2				
	A3				
	A4				
	A5	85	19	42,8	457
	A6				
	A7				
	mean	85,0	19,0	42,8	457,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B2				
	B3				
	B4				
	B5	68	7	4,4	152
	B6				
	B7				
	mean	68,0	7,0	4,4	152,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C2				
	C3				
	C4				
	C5	79	6	28,7	113
	C6				
	C7				
	mean	79,0	6,0	28,7	113,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	D1				
	D2				
	D3				
	D4				
	D5	107	7	42,7	211
	D6				
	D7				
	mean	107,0	7,0	42,7	211,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	E1				
	E2				
	E3				
	E4				
	E5	79	6	30,8	151
	E6				
	E7				
	mean	79,0	6,0	30,8	151,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	F1				
	F2				
	F3				
	F4				
	F5	100	7	29,1	186
	F6				
	F7				
	mean	100,0	7,0	29,1	186,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-11-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

Comments: E2 was weighed but the value was not recorded due to some distraction

Slice weights (n=6 or 7) **mg**

Treatment category (untreated, ne			Analysis category (histology n=9, triton n=6, iron n=12, MTT n=12)		
	sample	weight		sample	weight
A			histology		
	A2	28,8		A2	28,8
	A3	31,9		B2	25,0
	A4	26,3		C2	26,2
	A5	25,2		D1	25,6
	A6	25,3		D2	21,3
	A7	26,1		E1	23,4
	mean	27,3		E2	FALSE
	SD	2,6		F1	28,5
B	B2	25,0		F2	22,7
	B3	19,5		mean	25,2
	B4	22,2		SD	2,7
	B5	18,3	triton	A5	25,2
	B6	16,8		B5	18,3
	B7	17,0		C5	25,1
	mean	19,8		D5	21,2
	SD	3,2		E5	25,5
C	C2	26,2		F5	26,5
	C3	25,6		mean	23,6
	C4	21,6		SD	3,2
	C5	25,1	iron	A3	31,9
	C6	24,5		A4	26,3
	C7	20,3		B3	19,5
	mean	23,9		B4	22,2
	SD	2,4		C3	25,6
D	D1	25,6		C4	21,6
	D2	21,3		D3	22,6
	D3	22,6		D4	20,3
	D4	20,3		E3	24,0
	D5	21,2		E4	25,0
	D6	18,4		F3	26,9
	D7	16,1		F4	23,5
	mean	20,8		mean	24,1
	SD	3,0		SD	3,4
E	E1	23,4	MTT	A6	25,3
	E2	FALSE		A7	26,1
	E3	24,0		B6	16,8
	E4	25,0		B7	17,0
	E5	25,5		C6	24,5
	E6	21,7		C7	20,3
	E7	19,2		D6	18,4
	mean	23,1		D7	16,1
	SD	2,3		E6	21,7
F	F1	28,5		E7	19,2
	F2	22,7		F6	20,5
	F3	26,9		F7	18,6
	F4	23,5		mean	20,4
	F5	26,5		SD	3,4
	F6	20,5			
	F7	18,6			
	mean	23,9			
	SD	3,6			

EGN-11-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

MTT test (n=2; each with 2 parallels)

time	sample	OD-570	versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight**
0h	A6-1	1,287	0,051	87,6 %	
	A6-2	1,290	0,051	87,8 %	87,7 %
	A7-1	1,235	0,047	81,5 %	
	A7-2	1,219	0,047	80,4 %	81,0 %
	mean	1,248	0,049	84,3 %	84,3 %
	SD	0,037	0,002	3,9 %	4,8 %
24h	B6-1	1,096	0,065	112,3 %	
	B6-2	1,089	0,065	111,6 %	112,0 %
	B7-1	0,873	0,051	88,4 %	
	B7-2	0,865	0,051	87,6 %	88,0 %
	mean	0,942	0,058	100,0 %	100,0 %
	SD	0,127	0,008	13,8 %	16,9 %
24h	C6-1	0,503	0,021	35,4 %	
	C6-2	0,495	0,020	34,8 %	35,1 %
	C7-1	0,394	0,019	33,4 %	
	C7-2	0,391	0,019	33,2 %	33,3 %
	mean	0,427	0,020	34,2 %	34,2 %
	SD	0,059	0,001	1,1 %	1,3 %
24h	D6-1	0,945	0,051	88,4 %	
	D6-2	0,922	0,050	86,3 %	87,4 %
	D7-1	0,648	0,040	69,3 %	
	D7-2	0,626	0,039	67,0 %	68,1 %
	mean	0,732	0,045	77,7 %	77,7 %
	SD	0,165	0,006	11,2 %	13,6 %
24h	E6-1	0,748	0,034	59,4 %	
	E6-2	0,750	0,035	59,5 %	59,4 %
	E7-1	0,760	0,040	68,2 %	
	E7-2	0,758	0,039	68,0 %	68,1 %
	mean	0,754	0,037	63,8 %	63,8 %
	SD	0,006	0,003	5,0 %	6,1 %
24h	F6-1	0,746	0,036	62,7 %	
	F6-2	0,740	0,036	62,2 %	62,4 %
	F7-1	0,731	0,039	67,7 %	
	F7-2	0,728	0,039	67,4 %	67,5 %
	mean	0,736	0,038	65,0 %	65,0 %
	SD	0,008	0,002	3,0 %	3,6 %

EGN-11-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups:		Titles:	
	A (untreated / pre-inc.)		Culture media
	B (24h neg.ctrl.)		activities
	C (24h pos.ctrl.; menadione 200 uM)		leakage
	D (24h FeSO4 0.200 mM)		(n=4, 5, 6, or 7)
	E (24h FeSO4 1.00 mM)		U/L
	F (24h FeSO4 5.00 mM)		% of total

background: medium		4	0	0,1	43

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
24h	B2	124	20	28,4	1 209
	B3	138	19	36,7	1 150
	B4	109	17	23,1	1 157
	B5	104	15	23,9	922
	B6	77	12	17,4	657
	B7	95	13	20,7	839
	mean	107,8	16,0	25,0	989,0
	SD	21,5	3,2	6,8	219,0
24h	C2	104	13	15,6	1 594
	C3	121	14	19,5	1 493
	C4	81	12	10,5	1 301
	C5	128	13	20,3	1 618
	C6	116	10	17,3	1 389
	C7	88	7	12,2	1 220
	mean	106,3	11,5	15,9	1 435,8
	SD	18,8	2,6	3,9	160,3
24h	D1	120	20	26,4	1 366
	D2	128	21	28,8	1 346
	D3	121	19	26,3	1 285
	D4	72	13	14,1	773
	D5	97	19	20,2	1 038
	D6	101	16	26,3	860
	D7	92	14	21,0	779
	mean	104,4	17,4	23,3	1 063,9
	SD	19,8	3,1	5,1	267,0
24h	E1	139	22	34,0	1 424
	E2	202	25	57,6	1 688
	E3	129	24	27,5	1 120
	E4	205	28	49,8	1 629
	E5	190	28	48,4	1 583
	E6	131	23	29,8	1 270
	E7	93	17	19,9	970
	mean	155,6	23,9	38,1	1 383,4
	SD	43,4	3,8	13,9	273,2
24h	F1	240	31	71,8	1 448
	F2	261	39	71,8	781
	F3	322	35	88,8	1 359
	F4	187	29	47,4	1 043
	F5	201	30	55,9	1 394
	F6	144	21	35,2	789
	F7	162	22	43,6	826
	mean	216,7	29,6	59,2	1 091,4
	SD	61,9	6,5	19,0	303,0

Culture media ASAT activities (n=4, 5, 6, or 7) ASAT leakage (n=4, 5, 6, or 7) % of total ASAT
Culture media ALAT activities (n=4, 5, 6, or 7) ALAT leakage (n=4, 5, 6, or 7) % of total ALAT
Culture media GLDH activities (n=4, 5, 6, or 7 GLDH leakage (n=4, 5, 6, or 7) % of total GLDH
Culture media LDH activities (n=4, 5, 6, or 7) LDH leakage (n=4, 5, 6, or 7) % of total LDH

Triton supernatant enzyme activities (n=1)

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
	A2				
	A3				
	A4				
	A5	111	25	66,2	461
	A6				
	A7				
	mean	111,0	25,0	66,2	461,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B2				
	B3				
	B4				
	B5	106	8	38,1	206
	B6				
	B7				
	mean	106,0	8,0	38,1	206,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C2				
	C3				
	C4				
	C5	84	7	44,6	118
	C6				
	C7				
	mean	84,0	7,0	44,6	118,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	D1				
	D2				
	D3				
	D4				
	D5	120	8	48,2	194
	D6				
	D7				
	mean	120,0	8,0	48,2	194,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	E1				
	E2				
	E3				
	E4				
	E5	98	7	42,9	147
	E6				
	E7				
	mean	98,0	7,0	42,9	147,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	F1				
	F2				
	F3				
	F4				
	F5	77	6	34,8	128
	F6				
	F7				
	mean	77,0	6,0	34,8	128,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

EGN-12-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

Comments: B5 looked thick and large, and may have consisted of two or more parts sticking together. The recorded weight of 26,1 mg is not used any further.

Slice weights (n=6 or 7) **mg**

Treatment category (untreated, ne			Analysis category (histology n=9, triton n=6, iron n=12, MTT n=12)		
	sample	weight		sample	weight
A			histology		
	A2	27,5		A2	27,5
	A3	23,1		B2	19,6
	A4	23,9		C2	26,4
	A5	28,0		D1	20,2
	A6	24,8		D2	17,5
	A7	24,4		E1	20,3
	mean	25,3		E2	20,9
	SD	2,0		F1	20,8
B	B2	19,6		F2	20,5
	B3	18,6		mean	21,5
	B4	19,3		SD	3,3
	B5	FALSE	triton	A5	28,0
	B6	19,3		B5	FALSE
	B7	18,7		C5	22,1
	mean	19,1		D5	18,4
	SD	0,4		E5	19,5
C	C2	26,4		F5	21,9
	C3	25,4		mean	22,0
	C4	21,7		SD	3,7
	C5	22,1	iron	A3	23,1
	C6	23,4		A4	23,9
	C7	23,7		B3	18,6
	mean	23,8		B4	19,3
	SD	1,8		C3	25,4
D	D1	20,2		C4	21,7
	D2	17,5		D3	19,7
	D3	19,7		D4	18,8
	D4	18,8		E3	18,6
	D5	18,4		E4	17,8
	D6	19,4		F3	18,9
	D7	19,6		F4	19,9
	mean	19,1		mean	20,5
	SD	0,9		SD	2,5
E	E1	20,3	MTT	A6	24,8
	E2	20,9		A7	24,4
	E3	18,6		B6	19,3
	E4	17,8		B7	18,7
	E5	19,5		C6	23,4
	E6	20,5		C7	23,7
	E7	19,7		D6	19,4
	mean	19,6		D7	19,6
	SD	1,1		E6	20,5
F	F1	20,8		E7	19,7
	F2	20,5		F6	18,1
	F3	18,9		F7	20,3
	F4	19,9		mean	21,0
	F5	21,9		SD	2,4
	F6	18,1			
	F7	20,3			
	mean	20,1			
	SD	1,3			

EGN-12-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

MTT test (n=2; each with 2 parallels)

time	sample	OD-570	versus weight	and v. 24-h neg. (%)	**Abs.570nm / mg slice wet weight** slice
0h	A6-1	1,275	0,051	89,7 %	
	A6-2	1,234	0,050	86,9 %	88,3 %
	A7-1	1,071	0,044	76,6 %	
	A7-2	1,062	0,044	76,0 %	76,3 %
	mean	1,122	0,047	82,3 %	82,3 %
	SD	0,097	0,004	7,0 %	8,5 %
24h	B6-1	0,997	0,052	90,2 %	
	B6-2	0,988	0,051	89,4 %	89,8 %
	B7-1	1,182	0,063	110,3 %	
	B7-2	1,180	0,063	110,1 %	110,2 %
	mean	1,117	0,057	100,0 %	100,0 %
	SD	0,111	0,007	11,8 %	14,5 %
24h	C6-1	0,851	0,036	63,5 %	
	C6-2	0,839	0,036	62,6 %	63,0 %
	C7-1	0,772	0,033	56,9 %	
	C7-2	0,759	0,032	55,9 %	56,4 %
	mean	0,790	0,034	59,7 %	59,7 %
	SD	0,043	0,002	3,9 %	4,7 %
24h	D6-1	1,144	0,059	102,9 %	
	D6-2	1,149	0,059	103,4 %	103,2 %
	D7-1	1,095	0,056	97,5 %	
	D7-2	1,078	0,055	96,0 %	96,8 %
	mean	1,107	0,057	100,0 %	100,0 %
	SD	0,037	0,002	3,7 %	4,5 %
24h	E6-1	1,124	0,055	95,7 %	
	E6-2	1,106	0,054	94,2 %	94,9 %
	E7-1	1,206	0,061	106,9 %	
	E7-2	1,182	0,060	104,7 %	105,8 %
	mean	1,155	0,057	100,4 %	100,4 %
	SD	0,047	0,004	6,4 %	7,7 %
24h	F6-1	1,040	0,057	100,3 %	
	F6-2	1,042	0,058	100,5 %	100,4 %
	F7-1	0,911	0,045	78,3 %	
	F7-2	0,912	0,045	78,4 %	78,4 %
	mean	0,976	0,051	89,4 %	89,4 %
	SD	0,075	0,007	12,7 %	15,6 %

EGN-12-01: Effects of ferrous iron (Fe2+) on rat liver slices

Groups: A (untreated / pre-inc.)
B (24h neg.ctrl.)
C (24h pos.ctrl.; menadione 200 uM)
D (24h FeSO4 0.200 mM)
E (24h FeSO4 1.00 mM)
F (24h FeSO4 5.00 mM)

Titles: Culture media activities leakage (n=4, 5, 6, or 7) U/L % of total

background: medium		4	1	0,4	48

Culture media enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
24h	B2	56	11	14,5	409
	B3	62	10	15,1	451
	B4	54	8	12,2	363
	B5	133	22	38,7	920
	B6	80	15	22,8	512
	B7	61	10	15,7	368
	mean	74,3	12,7	19,8	503,8
	SD	30,2	5,1	9,9	211,3
24h	C2	63	16	5,0	806
	C3	65	12	5,1	937
	C4	107	12	17,2	1 156
	C5	74	14	6,8	986
	C6	86	15	13,7	643
	C7	87	14	9,5	943
	mean	80,3	13,8	9,6	911,8
	SD	16,5	1,6	5,0	173,3
24h	D1	47	12	10,0	352
	D2	103	18	28,1	628
	D3	90	12	23,6	541
	D4	49	11	13,2	301
	D5	67	14	20,8	422
	D6	73	14	14,3	577
	D7	82	17	22,4	559
	mean	73,0	14,0	18,9	482,9
	SD	20,6	2,6	6,5	124,5
24h	E1	110	21	29,1	836
	E2	100	18	22,2	570
	E3	91	17	21,1	516
	E4	105	18	25,4	553
	E5	103	18	27,4	665
	E6	105	15	26,5	637
	E7	103	16	27,7	541
	mean	102,4	17,6	25,6	616,9
	SD	5,9	1,9	3,0	110,3
24h	F1	194	23	65,8	666
	F2	171	21	55,6	500
	F3	171	20	62,6	438
	F4	172	21	62,3	460
	F5	201	26	63,1	639
	F6	165	15	59,1	526
	F7	198	23	61,5	625
	mean	181,7	21,3	61,4	550,6
	SD	15,2	3,4	3,3	91,9

Culture media ASAT activities (n=4, 5, 6, or 7) ASAT leakage (n=4, 5, 6, or 7) % of total ASAT
Culture media ALAT activities (n=4, 5, 6, or 7) ALAT leakage (n=4, 5, 6, or 7) % of total ALAT
Culture media GLDH activities (n=4, 5, 6, or 7 GLDH leakage (n=4, 5, 6, or 7) % of total GLDH
Culture media LDH activities (n=4, 5, 6, or 7) LDH leakage (n=4, 5, 6, or 7) % of total LDH

Triton supernatant enzyme activities (n=1)

Triton supernatant enzyme activities (U/L); raw data

time	sample	ASAT	ALAT	GLDH	LDH
0h					
	A2				
	A3				
	A4				
	A5	54	24	42,8	381
	A6				
	A7				
	mean	54,0	24,0	42,8	381,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	B2				
	B3				
	B4				
	B5	88	13	58,3	242
	B6				
	B7				
	mean	88,0	13,0	58,3	242,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	C2				
	C3				
	C4				
	C5	93	11	36,5	165
	C6				
	C7				
	mean	93,0	11,0	36,5	165,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	D1				
	D2				
	D3				
	D4				
	D5	72	15	19,3	130
	D6				
	D7				
	mean	72,0	15,0	19,3	130,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	E1				
	E2				
	E3				
	E4				
	E5	74	11	6,4	133
	E6				
	E7				
	mean	74,0	11,0	6,4	133,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
24h	F1				
	F2				
	F3				
	F4				
	F5	85	14	20,1	211
	F6				
	F7				
	mean	85,0	14,0	20,1	211,0
	SD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Nycomed Imaging AS

Analytical Sciences R&D, ICP-Lab.

Task number: **DEV 047.01.0036**
Journal no: -
Task: Fe and K in liver slices
Method: -
Task Origin: Edvard G. Nygaard
Project: NC100150
Date: 16-23/08/2001
Study: -
Sample ID: -

Apparatus:
* Perkin Elmer Optima 3300 DV ICP-AES, Perkin Elmer AS-91 Autosampler and HP Laserjet 2100.
* Laboratory balance, Mettler AT261 Delta Range,
with LC-P45 Printer, Investment no. 1004022, SOP FKA.058.15
*Autodiluter, Hamilton Microlab1000

* Finnpipette Digital, Labsystems:	40-200 µL	# -
	200-1000 µL	# ICP-3
	1-5 mL	# ICP-2

Reagents: * Spectrascan Element Standards for Atomic Spectroscopy, Teknolab AS, Drøbak, Norway

(A) 1000 µg Fe/mL	Prod.no. 8004	Lot no.	6
(B) 1000 µg K/mL	Prod.no. 8007	Lot no.	-
(C) 1000 µg Sc/mL	Prod.no. 8055	Lot no.	-

* (FeSO4:7H2O) Ferrous sulphate heptahydrate, Sigma (prod.no. F-2387)	Lot no:	-
* (HNO3) Nitric Acid 60%, Scanpure, Chem Scan AS, Elverum	Lot no:	-
* (HCl) Hydrochloric Acid 30%, Scanpure, Chem Scan AS, Elverum	Lot no:	-

Water: Milli Q Water system, purified with reverse osmosis and deionization, room 421, FoU II-4

Solutions:

(D) 100 µg Fe/mL and 100 µg K/mL:	Preparation date:	16.aug.01
10 mL (A) + 10 mL (B). Dilute to 100 mL with water	Reference:	-
(E) 10 µg Fe/mL and 10 µg K/mL:	Preparation date:	16.aug.01
10 mL (D). Dilute to 100 mL with water	Reference:	-
(F) 10 µg Sc/mL:	Preparation date:	16.aug.01
5 mL (C). Dilute to 500 mL with water	Reference:	-
(G) 100 µg Fe/mL:	Preparation date:	20.aug.01
10 mL (A). Dilute to 100 mL with water	Reference:	-
(H) ca. 20.0 mM ferrous sulphate equiv. ca. 1120 µg Fe/mL:	Preparation date:	20.aug.01
556 mg FeSO4:7H2O + 50 mL water + 10 mL HCl. Dilute to 100 mL with wa	Reference:	-
(J) ca. 2.00 mM ferrous sulphate equiv. ca. 112 µg Fe/mL:	Preparation date:	20.aug.01
10 mL (H). Dilute to 100 mL with water	Reference:	-

Standards:

Prepare according to table below.	Preparation date:	16.aug.01
Add 20 mL HNO3 + 10 mL HCl. Dilute to 100 mL with water	Reference:	-

	Conc Fe and K (µg/mL)	Vol A (mL)	Vol B (mL)	Vol D (mL)	Vol E (mL)	Vol F (mL)
std 0	-	-	-	-	-	10.00
std 0.5	0.50	-	-	-	5.00	10.00
std 1.0	1.00	-	-	-	10.00	10.00
std 5.0	5.00	-	-	5.00	-	10.00
std 20	20.00	-	-	20.00	-	10.00
std 50	50.00	5.00	5.00	-	-	10.00

Sample preparation: Each sample consists of ca. 25 mg rat liver and 0.5 mL water. Add 1 mL HNO3 + 0.5 mL HCl. Digest at 70 degrees overnight. Add 500 µL of 10 ppm Sc (sol. F). Dilute to ca. 5 mL with water.

Blank samples: **BL1-BL6:** 500 µL water

Control samples: 1577 b Bovine Liver NIST standard reference material
C1-C3: 50 mg of 1577b, suspended in 500 µL water.
C4-C6: 50 mg of 1577b, suspended in 500 µL water. Add 250 µL ca. 112 ppm Fe (sol. J).
C7-C9: 50 mg of 1577b, suspended in 500 µL water. Add 250 µL of 100 ppm Fe (sol. G).

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
03-01-A03	8/22/01	09:32:40	0,80165444	1,27E-02	1,57869977	Fe 259.939
03-01-B02	8/22/01	09:36:32	0,32227239	6,02E-03	1,86737493	Fe 259.939
03-01-B08	8/22/01	09:38:43	0,57808392	1,21E-02	2,0994641	Fe 259.939
03-01-B12	8/22/01	09:40:54	0,42849	6,97E-03	1,62593275	Fe 259.939
03-01-C03	8/22/01	09:43:06	0,59164738	9,03E-03	1,52658787	Fe 259.939
03-01-C10	8/22/01	09:45:18	0,51258636	9,72E-03	1,89533931	Fe 259.939
03-01-C15	8/22/01	09:47:31	0,43057517	7,63E-03	1,77096649	Fe 259.939
05-01-A03	8/22/01	09:51:25	0,33625448	7,96E-03	2,36775575	Fe 259.939
05-01-B02	8/22/01	09:53:39	0,36031213	6,12E-03	1,69961669	Fe 259.939
05-01-B08	8/22/01	09:55:53	0,4841025	3,28E-03	0,67718626	Fe 259.939
05-01-B12	8/22/01	09:58:04	0,31856756	7,14E-03	2,24120008	Fe 259.939
05-01-C03	8/22/01	10:00:11	0,35213505	4,34E-03	1,23107408	Fe 259.939
05-01-C10	8/22/01	10:02:19	0,49124274	8,21E-03	1,67031429	Fe 259.939
05-01-C15	8/22/01	10:06:09	0,42484426	1,23E-02	2,88948957	Fe 259.939
06-01-A03	8/22/01	10:08:18	0,33702002	7,89E-03	2,34058651	Fe 259.939
06-01-B03	8/22/01	10:10:27	0,39736805	8,48E-03	2,13390304	Fe 259.939
06-01-C03	8/22/01	10:12:37	0,22782059	3,57E-03	1,56746637	Fe 259.939
06-01-D03	8/22/01	10:14:47	0,3141152	6,86E-03	2,18261045	Fe 259.939
06-01-E03	8/22/01	10:16:58	0,30881231	6,49E-03	2,10169146	Fe 259.939
06-01-F03	8/22/01	10:20:50	0,34531324	6,67E-03	1,93159243	Fe 259.939
07-01-A03	8/22/01	10:23:02	0,50857431	1,12E-02	2,20561941	Fe 259.939
07-01-B03	8/22/01	10:25:14	0,20686756	6,77E-03	3,2724115	Fe 259.939
07-01-C03	8/22/01	10:27:27	0,25316172	6,03E-03	2,38068288	Fe 259.939
07-01-D03	8/22/01	10:29:40	0,6704904	1,22E-02	1,82333576	Fe 259.939
07-01-E03	8/22/01	10:31:54	0,45188775	8,26E-03	1,8271978	Fe 259.939
07-01-F03	8/22/01	10:35:50	0,49030677	8,15E-03	1,66170628	Fe 259.939
09-01-A03	8/22/01	10:49:24	0,54529294	1,15E-02	2,11047432	Fe 259.939
09-01-B03	8/22/01	10:51:33	0,2508092	6,42E-03	2,55929552	Fe 259.939
09-01-C03	8/22/01	10:53:44	0,28770898	6,39E-03	2,22243923	Fe 259.939
09-01-D03	8/22/01	10:55:54	0,24491237	2,97E-03	1,21318188	Fe 259.939
09-01-E03	8/22/01	10:58:06	0,37336947	1,03E-02	2,76021198	Fe 259.939
09-01-F03	8/22/01	11:00:17	0,69653597	1,36E-02	1,9516317	Fe 259.939
10-01-A03	8/22/01	11:04:11	0,41137342	7,86E-03	1,91108744	Fe 259.939
10-01-B03	8/22/01	11:06:24	0,41701711	6,04E-03	1,4472936	Fe 259.939
10-01-C03	8/22/01	11:08:39	0,31704823	6,25E-03	1,97217394	Fe 259.939
10-01-D03	8/22/01	11:10:52	0,35212312	7,89E-03	2,24172662	Fe 259.939
10-01-E03	8/22/01	11:13:02	0,57309938	9,08E-03	1,58464974	Fe 259.939
10-01-F03	8/22/01	11:15:08	0,7010514	1,29E-02	1,83359601	Fe 259.939
11-01-A03	8/22/01	11:18:57	0,69330394	9,23E-03	1,33147919	Fe 259.939
11-01-B03	8/22/01	11:21:04	0,29292758	3,71E-03	1,26614633	Fe 259.939
11-01-C03	8/22/01	11:23:12	0,33598559	8,78E-03	2,6125242	Fe 259.939
11-01-D03	8/22/01	11:25:20	0,40712361	5,73E-03	1,40657849	Fe 259.939
11-01-E03	8/22/01	11:27:29	0,4811932	7,10E-03	1,4763372	Fe 259.939
11-01-F03	8/22/01	11:29:38	0,85557781	1,21E-02	1,41115814	Fe 259.939
12-01-A03	8/22/01	11:33:29	0,51703871	9,05E-03	1,75032888	Fe 259.939
12-01-B03	8/22/01	11:35:40	0,21825986	4,86E-03	2,22734365	Fe 259.939
12-01-C03	8/22/01	11:37:52	0,43932525	9,28E-03	2,11189056	Fe 259.939
12-01-D03	8/22/01	11:40:03	0,28638698	8,19E-03	2,85839381	Fe 259.939
12-01-E03	8/22/01	11:42:15	0,52974003	7,57E-03	1,42926227	Fe 259.939
12-01-F03	8/22/01	11:44:27	1,08372233	2,03E-02	1,87242007	Fe 259.939
BL1	8/22/01	09:23:22	-2,74E-02	8,41E-04	3,06787843	Fe 259.939
BL2	8/22/01	09:30:29	-4,73E-02	2,71E-03	5,74355045	Fe 259.939
BL3	8/22/01	10:38:00	-5,00E-02	1,28E-03	2,5603649	Fe 259.939
BL4	8/22/01	10:45:33	-4,63E-02	1,31E-03	2,83557132	Fe 259.939
BL5	8/22/01	11:48:21	-5,04E-02	1,10E-03	2,17940367	Fe 259.939
BL6	8/22/01	11:55:58	-4,64E-02	1,10E-03	2,38076437	Fe 259.939
C1	8/22/01	09:25:01	3,01817209	4,63E-02	1,53477597	Fe 259.939
C2	8/22/01	10:40:08	1,91514503	1,15E-02	0,6002402	Fe 259.939
C3	8/22/01	11:50:34	1,90826719	8,31E-03	0,43557958	Fe 259.939

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
C4	8/22/01	09:26:40	7,52431531	7,64E-02	1,01513268	Fe 259.939
C5	8/22/01	10:41:46	7,667583	4,71E-02	0,61489877	Fe 259.939
C6	8/22/01	11:52:14	7,70793319	5,84E-02	0,75770278	Fe 259.939
C7	8/22/01	09:28:20	7,00672403	7,06E-02	1,00820562	Fe 259.939
C8	8/22/01	10:43:25	7,07676438	4,67E-02	0,6598984	Fe 259.939
C9	8/22/01	11:53:52	6,97201463	3,18E-02	0,45555359	Fe 259.939
std 5.0 µg/ml	8/22/01	09:21:13	5,0137206	5,50E-02	1,09742033	Fe 259.939
std 5.0 µg/ml	8/22/01	09:34:21	4,97784196	3,05E-02	0,61257038	Fe 259.939
std 5.0 µg/ml	8/22/01	09:49:13	4,98278006	2,47E-02	0,49593259	Fe 259.939
std 5.0 µg/ml	8/22/01	10:03:59	4,98411484	2,01E-02	0,40420497	Fe 259.939
std 5.0 µg/ml	8/22/01	10:18:39	4,99532256	2,26E-02	0,45217145	Fe 259.939
std 5.0 µg/ml	8/22/01	10:33:37	4,99062902	2,33E-02	0,46726221	Fe 259.939
std 5.0 µg/ml	8/22/01	10:47:14	4,99893178	2,58E-02	0,5154173	Fe 259.939
std 5.0 µg/ml	8/22/01	11:02:00	4,99678695	2,68E-02	0,53566546	Fe 259.939
std 5.0 µg/ml	8/22/01	11:16:48	4,99815587	2,29E-02	0,45907249	Fe 259.939
std 5.0 µg/ml	8/22/01	11:31:18	5,00311228	2,63E-02	0,52567466	Fe 259.939
std 5.0 µg/ml	8/22/01	11:46:09	5,00120273	2,82E-02	0,56340033	Fe 259.939
std 5.0 µg/ml	8/22/01	11:57:38	5,00274588	2,54E-02	0,5086743	Fe 259.939
03-01-A03	8/22/01	09:32:40	5,70945566	9,33E-02	1,63471272	K 766.490
03-01-B02	8/22/01	09:36:22	7,56881176	0,12225979	1,61531017	K 766.490
03-01-B08	8/22/01	09:38:43	9,56484243	0,20377378	2,13044577	K 766.490
03-01-B12	8/22/01	09:40:54	8,33668322	0,10735202	1,28770665	K 766.490
03-01-C03	8/22/01	09:43:06	6,77191256	8,09E-02	1,19484735	K 766.490
03-01-C10	8/22/01	09:45:18	7,12851167	0,17959069	2,51932934	K 766.490
03-01-C15	8/22/01	09:47:31	8,18529192	0,12922623	1,57876144	K 766.490
05-01-A03	8/22/01	09:51:25	4,13240886	4,26E-02	1,03112617	K 766.490
05-01-B02	8/22/01	09:53:39	10,0548956	0,13119523	1,30478958	K 766.490
05-01-B08	8/22/01	09:55:53	11,4339134	0,12693481	1,11016068	K 766.490
05-01-B12	8/22/01	09:57:54	7,5321783	7,00E-02	0,92871174	K 766.490
05-01-C03	8/22/01	10:00:01	7,97869395	5,99E-02	0,7511654	K 766.490
05-01-C10	8/22/01	10:02:19	10,6612161	0,19123535	1,79374799	K 766.490
05-01-C15	8/22/01	10:06:09	8,11053815	0,21017303	2,59135738	K 766.490
06-01-A03	8/22/01	10:08:18	5,82455346	7,90E-02	1,35654113	K 766.490
06-01-B03	8/22/01	10:10:17	9,86116405	0,12696183	1,28749329	K 766.490
06-01-C03	8/22/01	10:12:37	3,82230689	5,81E-02	1,51985175	K 766.490
06-01-D03	8/22/01	10:14:37	7,5506671	5,90E-02	0,78202465	K 766.490
06-01-E03	8/22/01	10:16:48	7,35492043	5,93E-02	0,80593435	K 766.490
06-01-F03	8/22/01	10:20:40	8,13123104	0,13939525	1,71431909	K 766.490
07-01-A03	8/22/01	10:23:02	3,88391393	4,17E-02	1,07347922	K 766.490
07-01-B03	8/22/01	10:25:14	3,41629122	6,66E-02	1,95061287	K 766.490
07-01-C03	8/22/01	10:27:27	2,73837054	4,35E-02	1,5887192	K 766.490
07-01-D03	8/22/01	10:29:40	8,61704278	0,11907634	1,38187009	K 766.490
07-01-E03	8/22/01	10:31:54	7,01150381	0,16590787	2,36622375	K 766.490
07-01-F03	8/22/01	10:35:50	7,0727675	9,24E-02	1,30577974	K 766.490
09-01-A03	8/22/01	10:49:24	4,45084426	6,68E-02	1,50183458	K 766.490
09-01-B03	8/22/01	10:51:33	4,01919805	4,91E-02	1,22203987	K 766.490
09-01-C03	8/22/01	10:53:44	3,50506569	4,55E-02	1,29878959	K 766.490
09-01-D03	8/22/01	10:55:54	4,67192523	7,47E-02	1,59910491	K 766.490
09-01-E03	8/22/01	10:58:06	2,00616444	3,76E-02	1,8735654	K 766.490
09-01-F03	8/22/01	11:00:17	2,02423357	1,83E-02	0,90462944	K 766.490
10-01-A03	8/22/01	11:04:11	6,86923756	8,76E-02	1,2749349	K 766.490
10-01-B03	8/22/01	11:06:24	9,06125964	9,64E-02	1,0640999	K 766.490
10-01-C03	8/22/01	11:08:39	5,27830723	8,53E-02	1,61533574	K 766.490
10-01-D03	8/22/01	11:10:52	5,50913101	0,10542907	1,91371502	K 766.490
10-01-E03	8/22/01	11:13:02	8,42210397	0,10460001	1,24197009	K 766.490
10-01-F03	8/22/01	11:15:08	6,32500674	9,42E-02	1,48924115	K 766.490
11-01-A03	8/22/01	11:18:57	5,45483873	7,78E-02	1,42625586	K 766.490
11-01-B03	8/22/01	11:21:04	5,22161505	4,95E-02	0,94825912	K 766.490
11-01-C03	8/22/01	11:23:12	4,35836878	5,72E-02	1,31215859	K 766.490

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
11-01-D03	8/22/01	11:25:20	5,8280932	5,92E-02	1,01642243	K 766.490
11-01-E03	8/22/01	11:27:29	6,21246828	6,85E-02	1,10207251	K 766.490
11-01-F03	8/22/01	11:29:38	2,68192921	9,40E-03	0,35054106	K 766.490
12-01-A03	8/22/01	11:33:29	6,29555609	0,10561002	1,67753286	K 766.490
12-01-B03	8/22/01	11:35:30	10,2729406	0,12495206	1,21632224	K 766.490
12-01-C03	8/22/01	11:37:52	7,46172849	0,16065105	2,1530005	K 766.490
12-01-D03	8/22/01	11:39:53	10,0884615	0,10559329	1,04667384	K 766.490
12-01-E03	8/22/01	11:42:15	8,98220948	0,16424096	1,82851404	K 766.490
12-01-F03	8/22/01	11:44:27	7,86470924	0,15426613	1,9614982	K 766.490
BL1	8/22/01	09:23:22	4,30E-02	1,31E-02	30,5049015	K 766.490
BL2	8/22/01	09:30:29	0,22684013	5,21E-02	22,9667457	K 766.490
BL3	8/22/01	10:38:00	8,94E-02	1,27E-02	14,1594602	K 766.490
BL4	8/22/01	10:45:33	0,21850596	6,27E-02	28,7081805	K 766.490
BL5	8/22/01	11:48:21	9,86E-02	1,24E-02	12,5433389	K 766.490
BL6	8/22/01	11:55:58	0,23272652	1,77E-02	7,6017322	K 766.490
C1	8/22/01	09:25:01	91,3606423	1,89201357	2,07092849	K 766.490
C2	8/22/01	10:39:58	93,5358148	0,49778944	0,53219127	K 766.490
C3	8/22/01	11:50:24	97,6601161	0,47727369	0,48870892	K 766.490
C4	8/22/01	09:26:40	92,2744127	1,42024261	1,53915107	K 766.490
C5	8/22/01	10:41:46	97,66531	1,14286284	1,17018298	K 766.490
C6	8/22/01	11:52:14	98,2462445	1,35812458	1,38236793	K 766.490
C7	8/22/01	09:28:20	97,0318661	1,4171053	1,46045352	K 766.490
C8	8/22/01	10:43:25	97,1646986	1,27657413	1,31382503	K 766.490
C9	8/22/01	11:53:52	91,5192714	0,92925479	1,01536515	K 766.490
std 5.0 µg/ml	8/22/01	09:21:13	5,02604386	7,33E-02	1,45832367	K 766.490
std 5.0 µg/ml	8/22/01	09:34:21	4,97994575	2,74E-02	0,54938308	K 766.490
std 5.0 µg/ml	8/22/01	09:49:13	4,93241094	2,40E-02	0,48607264	K 766.490
std 5.0 µg/ml	8/22/01	10:03:59	4,93445043	3,52E-02	0,71422116	K 766.490
std 5.0 µg/ml	8/22/01	10:18:39	4,88733094	3,39E-02	0,69347699	K 766.490
std 5.0 µg/ml	8/22/01	10:33:37	4,89639696	4,28E-02	0,87483084	K 766.490
std 5.0 µg/ml	8/22/01	10:47:14	4,93480241	6,76E-02	1,36999481	K 766.490
std 5.0 µg/ml	8/22/01	11:02:00	4,87937125	5,57E-02	1,14219375	K 766.490
std 5.0 µg/ml	8/22/01	11:16:48	4,8959648	5,12E-02	1,04488068	K 766.490
std 5.0 µg/ml	8/22/01	11:31:18	4,88051126	1,06E-02	0,21754362	K 766.490
std 5.0 µg/ml	8/22/01	11:46:09	4,85161922	3,97E-02	0,81896755	K 766.490
std 5.0 µg/ml	8/22/01	11:57:38	4,91378972	4,00E-02	0,81480229	K 766.490
03-01-A03	8/22/01	09:32:30	1,01937558	2,51E-02	2,46446735	Sc 424.683
03-01-B02	8/22/01	09:36:22	1,03394356	2,52E-02	2,43603799	Sc 424.683
03-01-B08	8/22/01	09:38:33	1,05176149	2,27E-02	2,15675452	Sc 424.683
03-01-B12	8/22/01	09:40:44	1,04006513	1,78E-02	1,70997205	Sc 424.683
03-01-C03	8/22/01	09:42:55	1,04438438	2,32E-02	2,22208734	Sc 424.683
03-01-C10	8/22/01	09:45:08	1,08446527	2,94E-02	2,70934791	Sc 424.683
03-01-C15	8/22/01	09:47:21	1,09513028	2,63E-02	2,40324603	Sc 424.683
05-01-A03	8/22/01	09:51:15	1,06956058	2,70E-02	2,5251903	Sc 424.683
05-01-B02	8/22/01	09:53:29	1,08213198	1,81E-02	1,67483627	Sc 424.683
05-01-B08	8/22/01	09:55:43	1,06509451	1,97E-02	1,85006753	Sc 424.683
05-01-B12	8/22/01	09:57:54	1,03731542	2,33E-02	2,24695129	Sc 424.683
05-01-C03	8/22/01	10:00:01	1,06124524	2,31E-02	2,17785216	Sc 424.683
05-01-C10	8/22/01	10:02:09	1,06219364	1,54E-02	1,45155343	Sc 424.683
05-01-C15	8/22/01	10:05:59	1,0974505	2,40E-02	2,18479661	Sc 424.683
06-01-A03	8/22/01	10:08:07	1,07890643	2,35E-02	2,17774097	Sc 424.683
06-01-B03	8/22/01	10:10:17	1,06365069	2,39E-02	2,24686849	Sc 424.683
06-01-C03	8/22/01	10:12:27	1,02887309	1,74E-02	1,68900278	Sc 424.683
06-01-D03	8/22/01	10:14:37	1,10700142	1,61E-02	1,45788744	Sc 424.683
06-01-E03	8/22/01	10:16:48	1,05619232	2,30E-02	2,17564952	Sc 424.683
06-01-F03	8/22/01	10:20:40	1,00378631	1,39E-02	1,38018776	Sc 424.683
07-01-A03	8/22/01	10:22:52	1,5189858	3,00E-02	1,97747877	Sc 424.683
07-01-B03	8/22/01	10:25:04	1,05572379	2,45E-02	2,31716773	Sc 424.683
07-01-C03	8/22/01	10:27:16	1,05028948	1,92E-02	1,82659372	Sc 424.683

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
07-01-D03	8/22/01	10:29:30	1,05413199	1,75E-02	1,66463341	Sc 424.683
07-01-E03	8/22/01	10:31:44	1,06502431	1,52E-02	1,42600702	Sc 424.683
07-01-F03	8/22/01	10:35:39	1,05408093	2,01E-02	1,90381625	Sc 424.683
09-01-A03	8/22/01	10:49:14	1,08348885	2,25E-02	2,07541677	Sc 424.683
09-01-B03	8/22/01	10:51:23	1,01263533	2,27E-02	2,24361581	Sc 424.683
09-01-C03	8/22/01	10:53:34	1,06382984	2,08E-02	1,95674274	Sc 424.683
09-01-D03	8/22/01	10:55:44	1,03146674	1,89E-02	1,83161961	Sc 424.683
09-01-E03	8/22/01	10:57:56	1,02806163	2,01E-02	1,95228521	Sc 424.683
09-01-F03	8/22/01	11:00:07	1,04040491	1,61E-02	1,54853054	Sc 424.683
10-01-A03	8/22/01	11:04:01	1,05567038	1,82E-02	1,72404968	Sc 424.683
10-01-B03	8/22/01	11:06:14	1,05018186	1,96E-02	1,86946771	Sc 424.683
10-01-C03	8/22/01	11:08:29	1,03924412	1,83E-02	1,76499551	Sc 424.683
10-01-D03	8/22/01	11:10:42	1,09207484	2,69E-02	2,46702697	Sc 424.683
10-01-E03	8/22/01	11:12:52	1,05696874	1,88E-02	1,78078374	Sc 424.683
10-01-F03	8/22/01	11:14:58	1,0061802	1,76E-02	1,75296967	Sc 424.683
11-01-A03	8/22/01	11:18:47	1,00830132	1,49E-02	1,47890539	Sc 424.683
11-01-B03	8/22/01	11:20:54	1,05109481	1,63E-02	1,55159315	Sc 424.683
11-01-C03	8/22/01	11:23:01	1,06270867	2,13E-02	2,00700882	Sc 424.683
11-01-D03	8/22/01	11:25:10	1,0294465	1,82E-02	1,77001705	Sc 424.683
11-01-E03	8/22/01	11:27:19	1,03639661	1,71E-02	1,650916	Sc 424.683
11-01-F03	8/22/01	11:29:28	1,021479	2,17E-02	2,12080919	Sc 424.683
12-01-A03	8/22/01	11:33:19	0,98175635	2,07E-02	2,11080837	Sc 424.683
12-01-B03	8/22/01	11:35:30	1,04925504	1,79E-02	1,70627356	Sc 424.683
12-01-C03	8/22/01	11:37:42	1,03198734	1,77E-02	1,71881688	Sc 424.683
12-01-D03	8/22/01	11:39:53	1,04363555	2,31E-02	2,21018103	Sc 424.683
12-01-E03	8/22/01	11:42:04	1,06681227	2,09E-02	1,95702898	Sc 424.683
12-01-F03	8/22/01	11:44:17	1,05522881	1,81E-02	1,71276168	Sc 424.683
BL1	8/22/01	09:23:12	1,0565515	1,86E-02	1,75710394	Sc 424.683
BL2	8/22/01	09:30:19	1,15370325	1,26E-02	1,09420126	Sc 424.683
BL3	8/22/01	10:37:50	1,08035032	1,56E-02	1,44829217	Sc 424.683
BL4	8/22/01	10:45:23	1,11104091	1,16E-02	1,04600094	Sc 424.683
BL5	8/22/01	11:48:11	1,09669611	1,61E-02	1,46579647	Sc 424.683
BL6	8/22/01	11:55:48	1,14421956	1,07E-02	0,93329062	Sc 424.683
C1	8/22/01	09:25:01	1,14926648	1,93E-02	1,67917978	Sc 424.683
C2	8/22/01	10:40:08	1,14491462	6,02E-03	0,52576483	Sc 424.683
C3	8/22/01	11:50:34	1,12656871	3,80E-03	0,33721861	Sc 424.683
C4	8/22/01	09:26:40	1,09590371	2,13E-02	1,94427729	Sc 424.683
C5	8/22/01	10:41:46	1,0792229	1,53E-02	1,41344734	Sc 424.683
C6	8/22/01	11:52:14	1,11112079	1,57E-02	1,41569194	Sc 424.683
C7	8/22/01	09:28:20	1,12821221	2,07E-02	1,83899995	Sc 424.683
C8	8/22/01	10:43:25	1,1009277	1,67E-02	1,52035679	Sc 424.683
C9	8/22/01	11:53:52	1,14853081	1,80E-02	1,56818096	Sc 424.683
std 5.0 µg/ml	8/22/01	09:21:13	1,05769573	2,14E-02	2,02304609	Sc 424.683
std 5.0 µg/ml	8/22/01	09:34:21	1,06996939	1,43E-02	1,33625974	Sc 424.683
std 5.0 µg/ml	8/22/01	09:49:13	1,07833797	1,18E-02	1,09544317	Sc 424.683
std 5.0 µg/ml	8/22/01	10:03:59	1,07136354	1,82E-02	1,70092701	Sc 424.683
std 5.0 µg/ml	8/22/01	10:18:39	1,07713539	1,79E-02	1,66056655	Sc 424.683
std 5.0 µg/ml	8/22/01	10:33:37	1,07437672	1,66E-02	1,54327276	Sc 424.683
std 5.0 µg/ml	8/22/01	10:47:14	1,07050789	1,58E-02	1,47320234	Sc 424.683
std 5.0 µg/ml	8/22/01	11:02:00	1,06943781	1,38E-02	1,29497548	Sc 424.683
std 5.0 µg/ml	8/22/01	11:16:48	1,06510639	1,91E-02	1,78997881	Sc 424.683
std 5.0 µg/ml	8/22/01	11:31:18	1,066709	1,72E-02	1,60845811	Sc 424.683
std 5.0 µg/ml	8/22/01	11:46:09	1,0731437	1,68E-02	1,56546868	Sc 424.683
std 5.0 µg/ml	8/22/01	11:57:38	1,07196935	1,59E-02	1,48681074	Sc 424.683

Nycomed Imaging AS

Analytical Sciences R&D, ICP-Lab.

Task number:	**DEV 047.01.0036**
Journal no:	-
Task:	Fe and K in liver slices
Task Origin:	Edvard G. Nygaard
Project:	NC100150
Date:	16-23/08/2001
Study:	-
Sample ID:	-
Parallel:	1.parallel, 22.8.01

Defined values:		**Measured values:**		
Total volume (mL)	5,00	500-µL autodispenser this day (mL)	0,4956	
Fe and K in std. 5 (µg/mL)	5,00	250-µL finnpipette this day (mL)	0,2503	
Sc in stock solution (µg/mL)	10,00	weighed in FeSO4;7H2O (mg)	553,7	
Fe in stock solution (µg/mL)	100,00			
Fe in std. bovine liver (µg/g)	184	**Computed values:**		
K in std. bovine liver (wt.%)	0,994	Sc as int.std. in samples (µg/mL)	0,9912	(based on autodispenser performance this day)
FeSO4;7H2O - Mw (g/mol)	278,01	conc. of Fe in FeSO4-sol. (µg/mL)	111,23	
Fe - Mw (g/mol)	55,847	added Fe from FeSO4-sol. (µg)	27,84	(based on finnpipette performance this day)
		added Fe from stock sol. (µg)	25,03	(based on finnpipette performance this day)
		K in std. bovine liver (µg/g)	9940	

Detection limits

	Fe 259.939			K 766.490		
Sample ID	Conc (Calib)	SD (Calib)	RSD (Conc)	Conc (Calib)	SD (Calib)	RSD (Conc)
BL1	-0,0274	0,0008	3,0679	0,0430	0,0131	30,5049
BL2	-0,0473	0,0027	5,7436	0,2268	0,0521	22,9667
BL3	-0,0500	0,0013	2,5604	0,0894	0,0127	14,1595
BL4	-0,0463	0,0013	2,8356	0,2185	0,0627	28,7082
BL5	-0,0504	0,0011	2,1794	0,0986	0,0124	12,5433
BL6	-0,0464	0,0011	2,3808	0,2327	0,0177	7,6017
Avg.	-0,0446			0,1515		
SD	0,0086	**DL:**	**0,1422**	0,0839	**DL:**	**1,3844**
%RSD	19,32 %	**QL:**	**0,4310**	55,38 %	**QL:**	**4,1951**

DL = 3.3 * SD * total volume QL = 10 * SD * total volume

Controls

		Fe 259.939			K 766.490		
Sample ID	Organ dry weight g	Analyte measured µg/mL	Analyte from organ µg/g	Total recovery %	Analyte measured µg/mL	Analyte from organ µg/g	Total recovery %
std 5.0 µg/mL	-	5,0137	-	100,27 %	5,0260	-	100,52 %
std 5.0 µg/mL	-	4,9778	-	99,56 %	4,9799	-	99,60 %
std 5.0 µg/mL	-	4,9828	-	99,66 %	4,9324	-	98,65 %
std 5.0 µg/mL	-	4,9841	-	99,68 %	4,9345	-	98,69 %
std 5.0 µg/mL	-	4,9953	-	99,91 %	4,8873	-	97,75 %
std 5.0 µg/mL	-	4,9906	-	99,81 %	4,8964	-	97,93 %
std 5.0 µg/mL	-	4,9989	-	99,98 %	4,9348	-	98,70 %
std 5.0 µg/mL	-	4,9968	-	99,94 %	4,8794	-	97,59 %
std 5.0 µg/mL	-	4,9982	-	99,96 %	4,8960	-	97,92 %
std 5.0 µg/mL	-	5,0031	-	100,06 %	4,8805	-	97,61 %
std 5.0 µg/mL	-	5,0012	-	100,02 %	4,8516	-	97,03 %
std 5.0 µg/mL	-	5,0027	-	100,05 %	4,9138	-	98,28 %
Avg.	-	4,9954	-	99,91 %	4,9177	-	98,35 %
SD	-	0,0101	-	0,20 %	0,0481	-	0,96 %
%RSD	-	0,20 %	-	0,20 %	0,98 %	-	0,98 %
C1	0,0484	3,0182	316,4044	171,96 %	91,3606	9 422,4318	94,79 %
C2	0,0506	1,9151	193,6529	105,25 %	93,5358	9 227,6989	92,83 %
C3	0,0512	1,9083	190,7119	103,65 %	97,6601	9 522,3256	95,80 %
C4	0,0481	7,5243	207,9902	103,15 %	92,2744	9 576,1861	96,34 %
C5	0,0515	7,6676	208,1683	103,34 %	97,6653	9 467,3600	95,25 %
C6	0,0511	7,7079	213,7460	104,08 %	98,2462	9 598,3114	96,56 %
C7	0,0502	7,0067	203,7198	102,89 %	97,0319	9 649,4387	97,08 %
C8	0,0508	7,0768	208,2073	103,58 %	97,1647	9 548,5430	96,06 %
C9	0,0491	6,9720	204,7492	102,99 %	91,5193	9 304,2535	93,60 %
Avg.	0,0501	5,6441	216,3722	111,21 %	95,1620	9 479,6166	95,37 %
SD	0,0013	2,5573	38,2220	22,79 %	2,9204	139,8498	1,41 %
%RSD	2,53 %	45,31 %	17,66 %	20,50 %	3,07 %	1,48 %	1,48 %

Nycomed Imaging AS

Analytical Sciences R&D, ICP-Lab.

Task number: **DEV 047.01.0036**
Journal no: -
Task: Fe and K in liver slices
Task Origin: Edvard G. Nygaard
Project: NC100150
Date: 16-23/08/2001
Study: -
Sample ID: -
Parallel: 1.parallel, 22.8.01

NB! Too much internal standard in sample 07-01-A03; New values computed manually

Samples		Fe 259.939			K 766.490			
	Organ wet weight	Analyte measured	Analyte from organ	Relative to pre-inc.	Analyte measured	Analyte from organ	Relative to pre-inc.	
Sample ID	g	µg/mL	µg/g	%	µg/mL	µg/g	%	
03-01-A03	0,0456	0,8017	92,7935	100,00 %	5,7095	609,4247	100,00 %	A: pre-incubated
03-01-B02	0,0214	0,3223	85,7231	92,38 %	7,5688	1 733,0163	284,37 %	B: 3-h negative
03-01-B08	0,0279	0,5781	111,5961	120,26 %	9,5648	1 686,9786	276,81 %	B: 6-h negative
03-01-B12	0,0209	0,4285	113,1848	121,97 %	8,3367	1 958,1773	321,32 %	B: 24-h negative
03-01-C03	0,0390	0,5916	81,5730	87,91 %	6,7719	848,7706	139,27 %	C: 3-h test 100
03-01-C10	0,0288	0,5126	96,7376	104,25 %	7,1285	1 211,2864	198,76 %	C: 6-h test 100
03-01-C15	0,0191	0,4306	124,3972	134,06 %	8,1853	2 103,0864	345,09 %	C: 24-h test 100
05-01-A03	0,0248	0,3363	76,7897	100,00 %	4,1324	802,6022	100,00 %	A: pre-incubated
05-01-B02	0,0244	0,3603	82,9784	108,06 %	10,0549	2 029,3839	252,85 %	B: 3-h negative
05-01-B08	0,0211	0,4841	125,2902	163,16 %	11,4339	2 673,5572	333,11 %	B: 6-h negative
05-01-B12	0,0185	0,3186	98,1594	127,83 %	7,5322	1 994,7774	248,54 %	B: 24-h negative
05-01-C03	0,0260	0,3521	76,2995	99,36 %	7,9787	1 505,2292	187,54 %	C: 3-h test 100
05-01-C10	0,0217	0,4912	123,4712	160,79 %	10,6612	2 421,5931	301,72 %	C: 6-h test 100
05-01-C15	0,0187	0,4248	125,5258	163,47 %	8,1105	2 128,0845	265,15 %	C: 24-h test 100
06-01-A03	0,0193	0,3370	98,8711	100,00 %	5,8246	1 469,7024	100,00 %	A: pre-incubated
06-01-B03	0,0166	0,3974	133,1296	134,65 %	9,8612	2 924,5970	198,99 %	B: 24-h negative
06-01-C03	0,0201	0,2278	67,7719	68,55 %	3,8223	913,1355	62,13 %	C: 24-h positive
06-01-D03	0,0199	0,3141	90,1350	91,16 %	7,5507	1 859,0867	126,49 %	D: 24-h test 100
06-01-E03	0,0164	0,3088	107,7545	108,98 %	7,3549	2 196,1641	149,43 %	E: 24-h test 200
06-01-F03	0,0161	0,3453	121,0980	122,48 %	8,1312	2 478,1767	168,62 %	F: 24-h test 400
07-01-A03	0,0270	**0,7460**	146,4152	100,00 %	**5,2369**	941,7404	100,00 %	A: pre-incubated
07-01-B03	0,0241	0,2069	52,1763	35,64 %	3,4163	677,3422	71,92 %	B: 24-h negative
07-01-C03	0,0336	0,2532	44,3131	30,27 %	2,7384	384,9507	40,88 %	C: 24-h positive
07-01-D03	0,0235	0,6705	152,1516	103,92 %	8,6170	1 801,1789	191,26 %	D: 24-h test 100
07-01-E03	0,0207	0,4519	119,9300	81,91 %	7,0115	1 657,0053	175,95 %	E: 24-h test 200
07-01-F03	0,0219	0,4903	122,1299	83,41 %	7,0728	1 580,1976	167,80 %	F: 24-h test 400
09-01-A03	0,0299	0,5453	98,6480	100,00 %	4,4508	718,9535	100,00 %	A: pre-incubated
09-01-B03	0,0251	0,2508	58,8509	59,66 %	4,0192	770,4574	107,16 %	B: 24-h negative
09-01-C03	0,0247	0,2877	67,2735	68,20 %	3,5051	678,8590	94,42 %	C: 24-h positive
09-01-D03	0,0204	0,2449	70,9644	71,94 %	4,6719	1 107,9469	154,11 %	D: 24-h test 0.200
09-01-E03	0,0280	0,3734	74,6414	75,66 %	2,0062	331,1897	46,07 %	E: 24-h test 1.00
09-01-F03	0,0253	0,6965	146,4740	148,48 %	2,0242	370,1050	51,48 %	F: 24-h test 5.00
10-01-A03	0,0263	0,4114	86,6912	100,00 %	6,8692	1 277,1360	100,00 %	A: pre-incubated
10-01-B03	0,0175	0,4170	131,8970	152,15 %	9,0613	2 545,6450	199,32 %	B: 24-h negative
10-01-C03	0,0222	0,3170	81,4573	93,96 %	5,2783	1 154,6859	90,41 %	C: 24-h positive
10-01-D03	0,0193	0,3521	102,7838	118,56 %	5,5091	1 387,9868	108,68 %	D: 24-h test 0.200
10-01-E03	0,0211	0,5731	146,3795	168,85 %	8,4221	1 959,8583	153,46 %	E: 24-h test 1.00
10-01-F03	0,0218	0,7011	171,0261	197,28 %	6,3250	1 415,9414	110,87 %	F: 24-h test 5.00
11-01-A03	0,0319	0,6933	115,6624	100,00 %	5,4548	831,2440	100,00 %	A: pre-incubated
11-01-B03	0,0195	0,2929	86,5512	74,83 %	5,2216	1 300,0290	156,40 %	B: 24-h negative
11-01-C03	0,0256	0,3360	74,3375	64,27 %	4,3584	821,6537	98,85 %	C: 24-h positive
11-01-D03	0,0226	0,4071	99,9438	86,41 %	5,8281	1 255,8830	151,08 %	D: 24-h test 0.200
11-01-E03	0,0240	0,4812	109,5449	94,71 %	6,2125	1 262,7013	151,91 %	E: 24-h test 1.00
11-01-F03	0,0269	0,8556	167,3234	144,67 %	2,6819	470,3396	56,58 %	F: 24-h test 5.00
12-01-A03	0,0231	0,5170	121,5716	100,00 %	6,2956	1 329,8818	100,00 %	A: pre-incubated
12-01-B03	0,0186	0,2183	70,6672	58,13 %	10,2729	2 720,8168	204,59 %	B: 24-h negative
12-01-C03	0,0254	0,4393	95,2653	78,36 %	7,4617	1 439,0210	108,21 %	C: 24-h positive
12-01-D03	0,0197	0,2864	84,0125	69,11 %	10,0885	2 522,0709	189,65 %	D: 24-h test 0.200
12-01-E03	0,0186	0,5297	154,3985	127,00 %	8,9822	2 373,8461	178,50 %	E: 24-h test 1.00
12-01-F03	0,0189	1,0837	298,5039	245,54 %	7,8647	2 040,5310	153,44 %	F: 24-h test 5.00

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
03-01-A04	8/23/01	14:30:08	0,32635902	9,81E-03	3,00573924	Fe 259.939
03-01-B03	8/23/01	14:34:00	0,37250378	5,89E-03	1,58251568	Fe 259.939
03-01-B09	8/23/01	14:36:11	-7,60E-02	1,54E-03	2,03145391	Fe 259.939
03-01-B13	8/23/01	14:38:23	0,49459975	1,12E-02	2,27139925	Fe 259.939
03-01-C04	8/23/01	14:40:36	0,51147528	1,16E-02	2,26863112	Fe 259.939
03-01-C11	8/23/01	14:42:48	0,45216341	6,62E-03	1,46344015	Fe 259.939
03-01-C16	8/23/01	14:45:02	0,31750545	1,81E-03	0,57089945	Fe 259.939
05-01-A04	8/23/01	14:48:56	0,22412544	5,33E-03	2,37866615	Fe 259.939
05-01-B03	8/23/01	14:51:10	0,29415488	1,24E-02	4,20243887	Fe 259.939
05-01-B09	8/23/01	14:53:24	0,43155138	8,27E-03	1,91540197	Fe 259.939
05-01-B13	8/23/01	14:55:35	0,30809642	7,19E-03	2,33435329	Fe 259.939
05-01-C04	8/23/01	14:57:42	0,33410897	7,48E-03	2,23902568	Fe 259.939
05-01-C11	8/23/01	14:59:51	0,44351184	1,18E-02	2,66781115	Fe 259.939
05-01-C16	8/23/01	15:15:12	0,36611623	8,92E-03	2,43702664	Fe 259.939
06-01-A04	8/23/01	15:17:21	0,36523443	6,78E-03	1,85702727	Fe 259.939
06-01-B04	8/23/01	15:19:30	0,37801997	9,24E-03	2,44551503	Fe 259.939
06-01-C04	8/23/01	15:21:40	0,22290251	3,74E-03	1,67888604	Fe 259.939
06-01-D04	8/23/01	15:23:50	0,25387914	6,87E-03	2,70479189	Fe 259.939
06-01-E04	8/23/01	15:26:00	0,35109266	8,06E-03	2,29588541	Fe 259.939
06-01-F04	8/23/01	15:29:53	0,32681732	8,66E-03	2,64994826	Fe 259.939
07-01-A04	8/23/01	15:32:04	0,47572341	8,44E-03	1,77424979	Fe 259.939
07-01-B04	8/23/01	15:34:17	0,29252415	7,64E-03	2,61198319	Fe 259.939
07-01-C04	8/23/01	15:36:29	0,25318791	3,81E-03	1,50460766	Fe 259.939
07-01-D04	8/23/01	15:38:43	0,3344642	8,33E-03	2,48943933	Fe 259.939
07-01-E04	8/23/01	15:40:56	0,24398404	4,81E-03	1,97226846	Fe 259.939
07-01-F04	8/23/01	15:44:53	0,52311025	1,80E-02	3,44645797	Fe 259.939
09-01-A04	8/23/01	15:58:29	0,59625483	1,82E-02	3,04734957	Fe 259.939
09-01-B04	8/23/01	16:00:39	0,18540838	6,34E-03	3,42104525	Fe 259.939
09-01-C04	8/23/01	16:02:49	0,13404901	3,97E-03	2,96238076	Fe 259.939
09-01-D04	8/23/01	16:04:59	0,27975375	6,47E-03	2,31297725	Fe 259.939
09-01-E04	8/23/01	16:07:10	0,41916504	1,17E-02	2,78007997	Fe 259.939
09-01-F04	8/23/01	16:09:21	0,71032215	1,61E-02	2,26745853	Fe 259.939
10-01-A04	8/23/01	16:13:14	0,48913479	9,46E-03	1,93392844	Fe 259.939
10-01-B04	8/23/01	16:15:27	0,33825708	8,77E-03	2,59345865	Fe 259.939
10-01-C04	8/23/01	16:17:40	0,2161822	5,32E-03	2,46084139	Fe 259.939
10-01-D04	8/23/01	16:19:53	0,31065207	8,07E-03	2,59910618	Fe 259.939
10-01-E04	8/23/01	16:22:03	0,37806169	7,66E-03	2,02570633	Fe 259.939
10-01-F04	8/23/01	16:24:09	0,45814381	1,02E-02	2,22021362	Fe 259.939
11-01-A04	8/23/01	16:27:57	0,38612522	9,96E-03	2,58015086	Fe 259.939
11-01-B04	8/23/01	16:30:04	0,33017141	9,71E-03	2,93995456	Fe 259.939
11-01-C04	8/23/01	16:32:12	0,24740683	4,23E-03	1,70964961	Fe 259.939
11-01-D04	8/23/01	16:34:20	0,36276535	1,24E-02	3,40625309	Fe 259.939
11-01-E04	8/23/01	16:36:29	0,43872315	8,59E-03	1,95896871	Fe 259.939
11-01-F04	8/23/01	16:38:08	1,27228765	1,13E-02	0,88684198	Fe 259.939
12-01-A04	8/23/01	16:42:00	0,37811456	8,89E-03	2,35025027	Fe 259.939
12-01-B04	8/23/01	16:44:11	0,24836631	1,02E-02	4,0868461	Fe 259.939
12-01-C04	8/23/01	16:46:22	0,21218309	3,74E-03	1,76417769	Fe 259.939
12-01-D04	8/23/01	16:48:33	0,44956473	1,45E-02	3,22919858	Fe 259.939
12-01-E04	8/23/01	16:50:45	0,49217747	8,78E-03	1,784152	Fe 259.939
12-01-F04	8/23/01	16:52:27	1,36779413	1,13E-02	0,82552964	Fe 259.939
BL1	8/23/01	14:20:18	-7,52E-02	2,67E-03	3,55269522	Fe 259.939
BL2	8/23/01	14:27:57	-7,17E-02	1,61E-03	2,24096238	Fe 259.939
BL3	8/23/01	15:47:04	-7,68E-02	9,16E-04	1,19222575	Fe 259.939
BL4	8/23/01	15:54:37	-7,38E-02	8,06E-04	1,09135752	Fe 259.939
BL5	8/23/01	16:56:21	-7,56E-02	9,37E-04	1,23943053	Fe 259.939
BL6	8/23/01	17:03:58	-7,33E-02	1,84E-03	2,50668265	Fe 259.939
C1	8/23/01	14:22:27	1,54961235	9,41E-03	0,60726226	Fe 259.939
C2	8/23/01	15:49:12	1,78938067	2,51E-02	1,40374125	Fe 259.939
C3	8/23/01	16:58:34	1,90592143	1,29E-02	0,67582924	Fe 259.939

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
C4	8/23/01	14:24:07	7,49669693	5,86E-02	0,78172493	Fe 259.939
C5	8/23/01	15:50:50	7,71268228	5,50E-02	0,71360383	Fe 259.939
C6	8/23/01	17:00:15	7,53885007	4,75E-02	0,63013111	Fe 259.939
C7	8/23/01	14:25:48	7,03365366	0,10393474	1,47767784	Fe 259.939
C8	8/23/01	15:52:29	6,8731285	4,88E-02	0,7101509	Fe 259.939
C9	8/23/01	17:01:52	7,13024472	4,92E-02	0,68987125	Fe 259.939
std 5.0 µg/ml	8/23/01	14:18:09	5,00604169	4,31E-02	0,86062092	Fe 259.939
std 5.0 µg/ml	8/23/01	14:31:49	4,99259956	2,92E-02	0,58515433	Fe 259.939
std 5.0 µg/ml	8/23/01	14:46:44	4,99416865	2,90E-02	0,58158673	Fe 259.939
std 5.0 µg/ml	8/23/01	15:01:32	4,99635609	1,84E-02	0,3677588	Fe 259.939
std 5.0 µg/ml	8/23/01	15:27:42	4,99995916	2,20E-02	0,43988022	Fe 259.939
std 5.0 µg/ml	8/23/01	15:42:40	5,00440137	2,37E-02	0,47370071	Fe 259.939
std 5.0 µg/ml	8/23/01	15:56:19	5,01300728	2,21E-02	0,44130369	Fe 259.939
std 5.0 µg/ml	8/23/01	16:11:03	5,00866644	2,03E-02	0,40430141	Fe 259.939
std 5.0 µg/ml	8/23/01	16:25:48	5,00907936	2,12E-02	0,42274873	Fe 259.939
std 5.0 µg/ml	8/23/01	16:39:49	5,01702416	2,29E-02	0,45645847	Fe 259.939
std 5.0 µg/ml	8/23/01	16:54:09	5,01006624	1,72E-02	0,34374778	Fe 259.939
std 5.0 µg/ml	8/23/01	17:05:38	5,0178834	2,82E-02	0,56128615	Fe 259.939
03-01-A04	8/23/01	14:30:08	5,01260176	0,10266409	2,04811977	K 766.490
03-01-B03	8/23/01	14:33:50	9,59487406	0,1755721	1,82985314	K 766.490
03-01-B09	8/23/01	14:36:11	5,44E-02	1,14E-02	20,9469319	K 766.490
03-01-B13	8/23/01	14:38:23	8,98433791	0,16305789	1,81491267	K 766.490
03-01-C04	8/23/01	14:40:36	8,93907273	0,14537543	1,62629203	K 766.490
03-01-C11	8/23/01	14:42:48	6,35249196	0,10975045	1,72767558	K 766.490
03-01-C16	8/23/01	14:45:02	4,47902041	2,04E-02	0,45613113	K 766.490
05-01-A04	8/23/01	14:48:56	5,47966844	0,10725672	1,9573578	K 766.490
05-01-B03	8/23/01	14:51:00	10,759347	0,14725624	1,36863546	K 766.490
05-01-B09	8/23/01	14:53:24	10,8104903	0,25130256	2,32461759	K 766.490
05-01-B13	8/23/01	14:55:24	8,66484734	0,11541576	1,33199991	K 766.490
05-01-C04	8/23/01	14:57:32	9,82614983	0,16808326	1,71057094	K 766.490
05-01-C11	8/23/01	14:59:51	9,28721016	0,21979174	2,36660667	K 766.490
05-01-C16	8/23/01	15:03:42	8,85739658	0,80808627	9,12329336	K 766.490
06-01-A04	8/23/01	15:17:21	4,55137506	7,96E-02	1,74925576	K 766.490
06-01-B04	8/23/01	15:19:20	9,83420084	0,19011772	1,93322999	K 766.490
06-01-C04	8/23/01	15:21:40	3,72264066	7,18E-02	1,92983132	K 766.490
06-01-D04	8/23/01	15:23:50	5,38797209	8,74E-02	1,62143959	K 766.490
06-01-E04	8/23/01	15:25:50	8,44693957	0,13879294	1,64311505	K 766.490
06-01-F04	8/23/01	15:29:43	8,25935038	0,12108446	1,46602889	K 766.490
07-01-A04	8/23/01	15:32:04	5,17294838	7,43E-02	1,43588122	K 766.490
07-01-B04	8/23/01	15:34:07	8,24847254	0,12409928	1,50451227	K 766.490
07-01-C04	8/23/01	15:36:29	3,59051917	5,65E-02	1,57334099	K 766.490
07-01-D04	8/23/01	15:38:33	6,8900459	0,13707908	1,98952351	K 766.490
07-01-E04	8/23/01	15:40:56	5,59704946	6,04E-02	1,07858713	K 766.490
07-01-F04	8/23/01	15:44:53	5,46084993	0,15611548	2,85881282	K 766.490
09-01-A04	8/23/01	15:58:29	4,13471031	8,49E-02	2,05379873	K 766.490
09-01-B04	8/23/01	16:00:39	3,76108879	0,10156856	2,70050941	K 766.490
09-01-C04	8/23/01	16:02:49	1,84400418	2,58E-02	1,40055161	K 766.490
09-01-D04	8/23/01	16:04:59	3,90980058	7,19E-02	1,83890174	K 766.490
09-01-E04	8/23/01	16:07:10	1,7763692	1,51E-02	0,84742106	K 766.490
09-01-F04	8/23/01	16:09:21	2,52190862	2,38E-02	0,94366001	K 766.490
10-01-A04	8/23/01	16:13:14	6,96978115	0,1168737	1,67686329	K 766.490
10-01-B04	8/23/01	16:15:17	8,09040874	0,10384441	1,28354962	K 766.490
10-01-C04	8/23/01	16:17:40	3,95169253	7,24E-02	1,83089141	K 766.490
10-01-D04	8/23/01	16:19:43	6,44768548	6,71E-02	1,04022644	K 766.490
10-01-E04	8/23/01	16:22:03	6,19969347	9,39E-02	1,51423531	K 766.490
10-01-F04	8/23/01	16:24:09	5,40693248	0,1286895	2,38008339	K 766.490
11-01-A04	8/23/01	16:27:57	4,26686921	8,89E-02	2,08340744	K 766.490
11-01-B04	8/23/01	16:30:04	6,14097637	0,14270967	2,32389213	K 766.490
11-01-C04	8/23/01	16:32:12	4,02912408	6,10E-02	1,51443451	K 766.490

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
11-01-D04	8/23/01	16:34:10	6,5073433	0,10716161	1,64677969	K 766.490
11-01-E04	8/23/01	16:36:29	5,02574924	8,28E-02	1,64762269	K 766.490
11-01-F04	8/23/01	16:38:08	5,1504262	7,06E-02	1,37020853	K 766.490
12-01-A04	8/23/01	16:42:00	6,19587104	0,12376315	1,99751016	K 766.490
12-01-B04	8/23/01	16:44:01	12,2415471	0,23387695	1,91051788	K 766.490
12-01-C04	8/23/01	16:46:22	4,20868316	8,75E-02	2,07843249	K 766.490
12-01-D04	8/23/01	16:48:33	11,322086	0,27578867	2,43584677	K 766.490
12-01-E04	8/23/01	16:50:45	7,67386081	0,11736924	1,52946789	K 766.490
12-01-F04	8/23/01	16:52:27	8,41687905	7,63E-02	0,90680347	K 766.490
BL1	8/23/01	14:20:18	1,32E-02	1,98E-02	150,342854	K 766.490
BL2	8/23/01	14:27:57	0,18745591	3,42E-02	18,2670185	K 766.490
BL3	8/23/01	15:47:04	1,22E-02	1,10E-02	90,3668435	K 766.490
BL4	8/23/01	15:54:37	0,19480772	4,05E-02	20,7875641	K 766.490
BL5	8/23/01	16:56:21	4,74E-02	3,11E-02	65,621275	K 766.490
BL6	8/23/01	17:03:58	0,18492412	4,21E-02	22,7429562	K 766.490
C1	8/23/01	14:22:17	85,1277026	0,77069017	0,90533416	K 766.490
C2	8/23/01	15:49:02	94,3878554	1,44830579	1,53441964	K 766.490
C3	8/23/01	16:58:24	102,040983	0,13038337	0,12777549	K 766.490
C4	8/23/01	14:24:07	101,975111	1,36283769	1,33644149	K 766.490
C5	8/23/01	15:50:50	101,947237	1,32635084	1,30101695	K 766.490
C6	8/23/01	17:00:15	96,4572913	1,11253675	1,1533983	K 766.490
C7	8/23/01	14:25:48	102,375798	1,93308498	1,88822458	K 766.490
C8	8/23/01	15:52:29	94,6903182	1,27273304	1,3441005	K 766.490
C9	8/23/01	17:01:52	102,81159	1,42976841	1,39066851	K 766.490
std 5.0 µg/ml	8/23/01	14:18:09	4,99898416	6,10E-02	1,21937669	K 766.490
std 5.0 µg/ml	8/23/01	14:31:49	4,97074775	8,21E-02	1,65265734	K 766.490
std 5.0 µg/ml	8/23/01	14:46:44	4,94395784	6,67E-03	0,13482254	K 766.490
std 5.0 µg/ml	8/23/01	15:01:32	4,92775962	2,97E-02	0,60259169	K 766.490
std 5.0 µg/ml	8/23/01	15:27:42	4,9138957	5,41E-02	1,1004893	K 766.490
std 5.0 µg/ml	8/23/01	15:42:40	4,89864788	4,20E-02	0,85674997	K 766.490
std 5.0 µg/ml	8/23/01	15:56:19	4,93525527	3,42E-02	0,69201466	K 766.490
std 5.0 µg/ml	8/23/01	16:11:03	4,90700677	3,76E-02	0,76710706	K 766.490
std 5.0 µg/ml	8/23/01	16:25:48	4,90681998	3,61E-02	0,7363879	K 766.490
std 5.0 µg/ml	8/23/01	16:39:49	4,89689523	4,81E-02	0,98199454	K 766.490
std 5.0 µg/ml	8/23/01	16:54:09	4,8631652	2,39E-02	0,491942	K 766.490
std 5.0 µg/ml	8/23/01	17:05:38	4,91086447	2,65E-02	0,53988153	K 766.490
03-01-A04	8/23/01	14:29:58	1,02986883	2,86E-02	2,77845187	Sc 424.683
03-01-B03	8/23/01	14:33:50	1,04006399	1,71E-02	1,64028757	Sc 424.683
03-01-B09	8/23/01	14:36:01	1,00443296	1,42E-02	1,41098461	Sc 424.683
03-01-B13	8/23/01	14:38:13	1,01218333	2,14E-02	2,11684882	Sc 424.683
03-01-C04	8/23/01	14:40:26	1,05502017	2,10E-02	1,98723713	Sc 424.683
03-01-C11	8/23/01	14:42:38	1,02108027	1,50E-02	1,47077099	Sc 424.683
03-01-C16	8/23/01	14:44:52	1,06216371	1,42E-02	1,33229111	Sc 424.683
05-01-A04	8/23/01	14:48:46	1,01828715	1,81E-02	1,77288869	Sc 424.683
05-01-B03	8/23/01	14:51:00	1,03067707	1,84E-02	1,78475317	Sc 424.683
05-01-B09	8/23/01	14:53:14	1,0675629	2,33E-02	2,18434418	Sc 424.683
05-01-B13	8/23/01	14:55:24	1,00863849	1,52E-02	1,51127181	Sc 424.683
05-01-C04	8/23/01	14:57:32	0,94459535	1,86E-02	1,9660481	Sc 424.683
05-01-C11	8/23/01	14:59:41	1,04430359	2,01E-02	1,92635078	Sc 424.683
05-01-C16	8/23/01	15:03:32	1,07388174	2,56E-02	2,3851021	Sc 424.683
06-01-A04	8/23/01	15:17:11	1,0489662	1,78E-02	1,69390807	Sc 424.683
06-01-B04	8/23/01	15:19:20	0,98932074	1,92E-02	1,94460342	Sc 424.683
06-01-C04	8/23/01	15:21:30	0,97939577	1,76E-02	1,79855002	Sc 424.683
06-01-D04	8/23/01	15:23:40	1,01510893	2,20E-02	2,17044338	Sc 424.683
06-01-E04	8/23/01	15:25:50	1,00592725	1,72E-02	1,70681927	Sc 424.683
06-01-F04	8/23/01	15:29:43	1,00430048	1,95E-02	1,93978245	Sc 424.683
07-01-A04	8/23/01	15:31:54	1,02013565	1,48E-02	1,44955591	Sc 424.683
07-01-B04	8/23/01	15:34:07	1,02768062	2,14E-02	2,08454805	Sc 424.683
07-01-C04	8/23/01	15:36:19	0,98750543	1,46E-02	1,47646918	Sc 424.683

Sample ID	Date	Time	Conc (Calib)	SD (Calib)	RSD (Conc)	Analyte Name
07-01-D04	8/23/01	15:38:33	1,08479709	2,36E-02	2,17962261	Sc 424.683
07-01-E04	8/23/01	15:40:46	0,96959977	1,79E-02	1,85045409	Sc 424.683
07-01-F04	8/23/01	15:44:43	1,0674398	2,20E-02	2,06176682	Sc 424.683
09-01-A04	8/23/01	15:58:19	1,04218065	2,31E-02	2,22103783	Sc 424.683
09-01-B04	8/23/01	16:00:28	1,05715237	2,75E-02	2,59874914	Sc 424.683
09-01-C04	8/23/01	16:02:39	1,02023845	2,65E-02	2,59309106	Sc 424.683
09-01-D04	8/23/01	16:04:49	1,0317032	2,20E-02	2,12899997	Sc 424.683
09-01-E04	8/23/01	16:07:00	0,98639387	2,03E-02	2,05897829	Sc 424.683
09-01-F04	8/23/01	16:09:11	1,01898491	2,22E-02	2,18209309	Sc 424.683
10-01-A04	8/23/01	16:13:04	1,00122	1,21E-02	1,20898275	Sc 424.683
10-01-B04	8/23/01	16:15:17	1,02395018	1,95E-02	1,90182903	Sc 424.683
10-01-C04	8/23/01	16:17:29	1,05141154	2,30E-02	2,19006453	Sc 424.683
10-01-D04	8/23/01	16:19:43	1,05834997	2,09E-02	1,97926353	Sc 424.683
10-01-E04	8/23/01	16:21:53	1,00329543	1,80E-02	1,79298593	Sc 424.683
10-01-F04	8/23/01	16:23:59	0,97276696	1,92E-02	1,97113548	Sc 424.683
11-01-A04	8/23/01	16:27:47	1,01487286	2,07E-02	2,04035112	Sc 424.683
11-01-B04	8/23/01	16:29:54	1,03586971	2,46E-02	2,3749023	Sc 424.683
11-01-C04	8/23/01	16:32:02	1,01800753	2,34E-02	2,30067958	Sc 424.683
11-01-D04	8/23/01	16:34:10	1,00738911	2,34E-02	2,31824265	Sc 424.683
11-01-E04	8/23/01	16:36:19	1,0000395	2,27E-02	2,27162813	Sc 424.683
11-01-F04	8/23/01	16:38:08	0,96750997	1,76E-02	1,82206148	Sc 424.683
12-01-A04	8/23/01	16:41:50	1,04838757	1,96E-02	1,86859665	Sc 424.683
12-01-B04	8/23/01	16:44:01	1,00658482	2,37E-02	2,35084974	Sc 424.683
12-01-C04	8/23/01	16:46:11	1,01275129	1,62E-02	1,60236066	Sc 424.683
12-01-D04	8/23/01	16:48:23	1,02159992	2,32E-02	2,27492162	Sc 424.683
12-01-E04	8/23/01	16:50:34	0,99700724	1,94E-02	1,94163266	Sc 424.683
12-01-F04	8/23/01	16:52:27	1,00326024	1,73E-02	1,72122105	Sc 424.683
BL1	8/23/01	14:20:08	1,01480552	1,82E-02	1,79628327	Sc 424.683
BL2	8/23/01	14:27:47	1,04230833	2,26E-02	2,1721845	Sc 424.683
BL3	8/23/01	15:46:54	0,97540599	2,03E-02	2,08354571	Sc 424.683
BL4	8/23/01	15:54:27	1,03856977	1,86E-02	1,79267504	Sc 424.683
BL5	8/23/01	16:56:11	1,00808055	1,86E-02	1,85000357	Sc 424.683
BL6	8/23/01	17:03:48	1,03772627	1,31E-02	1,25824911	Sc 424.683
C1	8/23/01	14:22:27	1,03843506	6,77E-03	0,65153763	Sc 424.683
C2	8/23/01	15:49:12	0,99610164	1,09E-02	1,09288116	Sc 424.683
C3	8/23/01	16:58:34	1,01174223	5,89E-03	0,5824479	Sc 424.683
C4	8/23/01	14:24:07	1,04543337	1,35E-02	1,29318584	Sc 424.683
C5	8/23/01	15:50:50	1,00807287	1,74E-02	1,72536391	Sc 424.683
C6	8/23/01	17:00:15	1,01613597	1,53E-02	1,50582551	Sc 424.683
C7	8/23/01	14:25:48	1,05147125	2,09E-02	1,98461501	Sc 424.683
C8	8/23/01	15:52:29	1,03733937	1,72E-02	1,66132578	Sc 424.683
C9	8/23/01	17:01:52	0,98764246	2,27E-02	2,3012252	Sc 424.683
std 5.0 µg/ml	8/23/01	14:18:09	1,05258214	1,61E-02	1,53303947	Sc 424.683
std 5.0 µg/ml	8/23/01	14:31:49	1,0593165	1,74E-02	1,64439398	Sc 424.683
std 5.0 µg/ml	8/23/01	14:46:44	1,06221174	1,90E-02	1,78792999	Sc 424.683
std 5.0 µg/ml	8/23/01	15:01:32	1,06143388	1,31E-02	1,23633684	Sc 424.683
std 5.0 µg/ml	8/23/01	15:27:42	1,06117549	1,18E-02	1,11124642	Sc 424.683
std 5.0 µg/ml	8/23/01	15:42:40	1,06228665	1,23E-02	1,1619939	Sc 424.683
std 5.0 µg/ml	8/23/01	15:56:19	1,04755743	1,95E-02	1,86354936	Sc 424.683
std 5.0 µg/ml	8/23/01	16:11:03	1,04996728	1,64E-02	1,56669446	Sc 424.683
std 5.0 µg/ml	8/23/01	16:25:48	1,04387233	1,94E-02	1,86238985	Sc 424.683
std 5.0 µg/ml	8/23/01	16:39:49	1,04382521	1,88E-02	1,79733868	Sc 424.683
std 5.0 µg/ml	8/23/01	16:54:09	1,04516426	1,64E-02	1,56562865	Sc 424.683
std 5.0 µg/ml	8/23/01	17:05:38	1,04160179	1,62E-02	1,55951992	Sc 424.683
05-01-C16	8/23/01	15:03:42	0,34600704	3,89E-02	11,2399762	Fe 259.939

Nycomed Imaging AS

Analytical Sciences R&D, ICP-Lab.

Task number:	**DEV 047.01.0036**
Journal no:	-
Task:	Fe and K in liver slices
Task Origin:	Edvard G. Nygaard
Project:	NC100150
Date:	16-23/08/2001
Study:	-
Sample ID:	-
Parallel:	2.parallel, 23.8.01

Defined values:		**Measured values:**		
Total volume (mL)	5,00	500-µL autodispenser this day (mL)	0,4966	
Fe and K in std. 5 (µg/mL)	5,00	250-µL finnpipette this day (mL)	0,2505	
Sc in stock solution (µg/mL)	10,00	weighed in FeSO4;7H2O (mg)	553,7	
Fe in stock solution (µg/mL)	100,00			
Fe in std. bovine liver (µg/g)	184	**Computed values:**		
K in std. bovine liver (wt.%)	0,994	Sc as int.std. in samples (µg/mL)	0,9932	(based on autodispenser performance this day)
FeSO4;7H2O - Mw (g/mol)	278,01	conc. of Fe in FeSO4-sol. (µg/mL)	111,23	
Fe - Mw (g/mol)	55,847	added Fe from FeSO4-sol. (µg)	27,86	(based on finnpipette performance this day)
		added Fe from stock sol. (µg)	25,05	(based on finnpipette performance this day)
		K in std. bovine liver (µg/g)	9940	

Detection limits

	Fe 259.939			K 766.490		
Sample ID	Conc (Calib)	SD (Calib)	RSD (Conc)	Conc (Calib)	SD (Calib)	RSD (Conc)
BL1	-0,0752	0,0027	3,5527	0,0132	0,0198	150,3429
BL2	-0,0717	0,0016	2,2410	0,1875	0,0342	18,2670
BL3	-0,0768	0,0009	1,1922	0,0122	0,0110	90,3668
BL4	-0,0738	0,0008	1,0914	0,1948	0,0405	20,7876
BL5	-0,0756	0,0009	1,2394	0,0474	0,0311	65,6213
BL6	-0,0733	0,0018	2,5067	0,1849	0,0421	22,7430
Avg.	-0,0744			0,1067		
SD	0,0018	**DL:**	**0,0305**	0,0912	**DL:**	**1,5050**
%RSD	2,48 %	**QL:**	**0,0923**	85,51 %	**QL:**	**4,5605**

DL = 3.3 * SD * total volume QL = 10 * SD * total volume

Controls

		Fe 259.939			K 766.490		
Sample ID	Organ dry weight g	Analyte measured µg/mL	Analyte from organ µg/g	Total recovery %	Analyte measured µg/mL	Analyte from organ µg/g	Total recovery %
std 5.0 µg/mL	-	5,0060	-	100,12 %	4,9990	-	99,98 %
std 5.0 µg/mL	-	4,9926	-	99,85 %	4,9707	-	99,41 %
std 5.0 µg/mL	-	4,9942	-	99,88 %	4,9440	-	98,88 %
std 5.0 µg/mL	-	4,9964	-	99,93 %	4,9278	-	98,56 %
std 5.0 µg/mL	-	5,0000	-	100,00 %	4,9139	-	98,28 %
std 5.0 µg/mL	-	5,0044	-	100,09 %	4,8986	-	97,97 %
std 5.0 µg/mL	-	5,0130	-	100,26 %	4,9353	-	98,71 %
std 5.0 µg/mL	-	5,0087	-	100,17 %	4,9070	-	98,14 %
std 5.0 µg/mL	-	5,0091	-	100,18 %	4,9068	-	98,14 %
std 5.0 µg/mL	-	5,0170	-	100,34 %	4,8969	-	97,94 %
std 5.0 µg/mL	-	5,0101	-	100,20 %	4,8632	-	97,26 %
std 5.0 µg/mL	-	5,0179	-	100,36 %	4,9109	-	98,22 %
Avg.	-	5,0058	-	100,12 %	4,9228	-	98,46 %
SD	-	0,0085	-	0,17 %	0,0360	-	0,72 %
%RSD	-	0,17 %	-	0,17 %	0,73 %	-	0,73 %
C1	0,0443	1,5496	183,2975	99,62 %	85,1277	9 596,0544	96,54 %
C2	0,0501	1,7894	186,0064	101,09 %	94,3879	9 409,3009	94,66 %
C3	0,0537	1,9059	184,3878	100,21 %	102,0410	9 491,0914	95,48 %
C4	0,0514	7,4967	194,4143	101,43 %	101,9751	9 909,3823	99,69 %
C5	0,0533	7,7127	207,7453	103,36 %	101,9472	9 553,5250	96,11 %
C6	0,0501	7,5389	203,6659	102,66 %	96,4573	9 615,8314	96,74 %
C7	0,0519	7,0337	202,1249	102,72 %	102,3758	9 852,5180	99,12 %
C8	0,0494	6,8731	196,1064	101,75 %	94,6903	9 573,2447	96,31 %
C9	0,0531	7,1302	206,6523	103,45 %	102,8116	9 670,8973	97,29 %
Avg.	0,0508	5,4478	196,0445	101,81 %	97,9793	9 630,2050	96,88 %
SD	0,0029	2,7884	9,6584	1,35 %	5,9381	161,1161	1,62 %
%RSD	5,68 %	51,18 %	4,93 %	1,33 %	6,06 %	1,67 %	1,67 %

Nycomed Imaging AS

Analytical Sciences R&D, ICP-Lab.

Task number: **DEV 047.01.0036**
Journal no: -
Task: Fe and K in liver slices
Task Origin: Edvard G. Nygaard
Project: NC100150
Date: 16-23/08/2001
Study: -
Sample ID: -
Parallel: 2.parallel, 23.8.01

NB! Missing sample no. 03-01-B09; All values FALSE

Samples		Fe 259.939			K 766.490			
	Organ wet weight	Analyte measured	Analyte from organ	Relative to pre-inc.	Analyte measured	Analyte from organ	Relative to pre-inc.	
Sample ID	g	µg/mL	µg/g	%	µg/mL	µg/g	%	
03-01-A04	0,0225	0,3264	89,0583	100,00 %	5,0126	1 090,2091	100,00 %	A: pre-incubated
03-01-B03	0,0215	0,3725	103,9318	116,70 %	9,5949	2 206,5612	202,40 %	B: 3-h negative
03-01-B09	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	B: 6-h negative
03-01-B13	0,0161	0,4946	176,7090	198,42 %	8,9843	2 757,0426	252,89 %	B: 24-h negative
03-01-C04	0,0204	0,5115	143,5977	161,24 %	8,9391	2 164,8068	198,57 %	C: 3-h test 100
03-01-C11	0,0274	0,4522	96,0888	107,89 %	6,3525	1 139,7502	104,54 %	C: 6-h test 100
03-01-C16	0,0211	0,3175	92,8693	104,28 %	4,4790	1 036,1042	95,04 %	C: 24-h test 100
05-01-A04	0,0208	0,2241	71,7617	100,00 %	5,4797	1 291,5884	100,00 %	A: pre-incubated
05-01-B03	0,0235	0,2942	78,4166	109,27 %	10,7593	2 266,5290	175,48 %	B: 3-h negative
05-01-B09	0,0220	0,4316	114,9897	160,24 %	10,8105	2 432,6885	188,35 %	B: 6-h negative
05-01-B13	0,0180	0,3081	106,2499	148,06 %	8,6648	2 377,2740	184,06 %	B: 24-h negative
05-01-C04	0,0227	0,3341	89,9806	125,39 %	9,8261	2 140,8566	165,75 %	C: 3-h test 100
05-01-C11	0,0272	0,4435	95,2050	132,67 %	9,2872	1 687,6010	130,66 %	C: 6-h test 100
05-01-C16	0,0193	0,3661	114,1242	159,03 %	8,8574	2 267,0300	175,52 %	C: 24-h test 100
06-01-A04	0,0171	0,3652	128,5490	100,00 %	4,5514	1 299,6241	100,00 %	A: pre-incubated
06-01-B04	0,0187	0,3780	120,9688	94,10 %	9,8342	2 600,9465	200,13 %	B: 24-h negative
06-01-C04	0,0203	0,2229	73,2280	56,97 %	3,7226	890,6354	68,53 %	C: 24-h positive
06-01-D04	0,0133	0,2539	123,4144	96,01 %	5,3880	1 985,4554	152,77 %	D: 24-h test 100
06-01-E04	0,0195	0,3511	109,1015	84,87 %	8,4469	2 138,5330	164,55 %	E: 24-h test 200
06-01-F04	0,0156	0,3268	128,5963	100,04 %	8,2594	2 613,0415	201,06 %	F: 24-h test 400
07-01-A04	0,0264	0,4757	104,1906	100,00 %	5,1729	959,5242	100,00 %	A: pre-incubated
07-01-B04	0,0215	0,2925	85,3319	81,90 %	8,2485	1 893,4446	197,33 %	B: 24-h negative
07-01-C04	0,0328	0,2532	49,9377	47,93 %	3,5905	531,0760	55,35 %	C: 24-h positive
07-01-D04	0,0239	0,3345	85,5371	82,10 %	6,8900	1 419,1182	147,90 %	D: 24-h test 100
07-01-E04	0,0162	0,2440	98,2677	94,32 %	5,5970	1 694,5644	176,60 %	E: 24-h test 200
07-01-F04	0,0231	0,5231	129,3319	124,13 %	5,4608	1 158,9154	120,78 %	F: 24-h test 400
09-01-A04	0,0334	0,5963	100,3979	100,00 %	4,1347	603,0014	100,00 %	A: pre-incubated
09-01-B04	0,0243	0,1854	53,4592	53,25 %	3,7611	751,9399	124,70 %	B: 24-h negative
09-01-C04	0,0288	0,1340	36,1896	36,05 %	1,8440	301,6221	50,02 %	C: 24-h positive
09-01-D04	0,0248	0,2798	71,4026	71,12 %	3,9098	766,7621	127,16 %	D: 24-h test 0.200
09-01-E04	0,0295	0,4192	83,6556	83,32 %	1,7764	283,0014	46,93 %	E: 24-h test 1.00
09-01-F04	0,0259	0,7103	151,4914	150,89 %	2,5219	466,2641	77,32 %	F: 24-h test 5.00
10-01-A04	0,0287	0,4891	98,1773	100,00 %	6,9698	1 195,6656	100,00 %	A: pre-incubated
10-01-B04	0,0195	0,3383	105,8103	107,77 %	8,0904	2 047,1149	171,21 %	B: 24-h negative
10-01-C04	0,0244	0,2162	59,5462	60,65 %	3,9517	787,9163	65,90 %	C: 24-h positive
10-01-D04	0,0208	0,3107	92,5614	94,28 %	6,4477	1 524,2848	127,48 %	D: 24-h test 0.200
10-01-E04	0,0182	0,3781	124,3035	126,61 %	6,1997	1 673,9101	140,00 %	E: 24-h test 1.00
10-01-F04	0,0187	0,4581	142,3922	145,04 %	5,4069	1 417,1849	118,53 %	F: 24-h test 5.00
11-01-A04	0,0263	0,3861	87,5529	100,00 %	4,2669	790,9141	100,00 %	A: pre-incubated
11-01-B04	0,0222	0,3302	91,1204	104,07 %	6,1410	1 359,0801	171,84 %	B: 24-h negative
11-01-C04	0,0216	0,2474	74,4931	85,08 %	4,0291	907,9776	114,80 %	C: 24-h positive
11-01-D04	0,0203	0,3628	107,6770	122,99 %	6,5073	1 576,5228	199,33 %	D: 24-h test 0.200
11-01-E04	0,0250	0,4387	102,6253	117,22 %	5,0257	983,8177	124,39 %	E: 24-h test 1.00
11-01-F04	0,0235	1,2723	286,5300	327,26 %	5,1504	1 073,1416	135,68 %	F: 24-h test 5.00
12-01-A04	0,0239	0,3781	94,6690	100,00 %	6,1959	1 273,8934	100,00 %	A: pre-incubated
12-01-B04	0,0193	0,2484	83,6190	88,33 %	12,2415	3 143,7529	246,78 %	B: 24-h negative
12-01-C04	0,0217	0,2122	66,0337	69,75 %	4,2087	945,1664	74,20 %	C: 24-h positive
12-01-D04	0,0188	0,4496	139,3532	147,20 %	11,3221	2 982,8259	234,15 %	D: 24-h test 0.200
12-01-E04	0,0178	0,4922	159,1519	168,11 %	7,6739	2 125,6180	166,86 %	E: 24-h test 1.00
12-01-F04	0,0199	1,3678	362,3611	382,77 %	8,4169	2 087,9945	163,91 %	F: 24-h test 5.00

Nycomed Imaging AS

Analytical Sciences R&D, ICP-Lab.

Task number: **DEV 047.01.0036**
Journal no: -
Task: Fe and K in liver slices
Task Origin: Edvard G. Nygaard
Project: NC100150
Date: 16-23/08/2001
Study: -
Sample ID: -

Analysed by: Eva Mokastet and Edvard G. Nygaard Sign:_____Date:_____
Controlled by: Eva Mokastet Sign:_____Date:_____

Results:

µg/g is content of Fe or K per slice wet weight; % is content relative to pre-incubated slices from the same experiments.
Identical experiments: {03, 05 (3-6-24-h, 100)}, {06, 07 (24-h, 100/200/400)}, {09 to 12 (24-h, 200/1000/5000)}

		Fe 259.939			K 766.490		
Groups	**no. of slices**	(µg/g)	SD	(%)	(µg/g)	SD	(%)
03, 05	4	82,60	9,95	100,00 %	948,46	302,24	100,00 %
06, 07	4	119,51	22,11	100,00 %	1167,65	260,13	100,00 %
09 to 12	8	100,42	12,39	100,00 %	1002,59	294,63	100,00 %
Pre-incubated total	16	100,74		100,00 %	1030,32		100,00 %
06, 07, 09 to 12	12	106,78		100,00 %	1057,61		100,00 %
03, 05, 06, 07	8	101,05		100,00 %	1058,05		100,00 %
3-h negative (03, 05)	4	87,76	11,19	106,25 %	2058,87	239,43	217,08 %
6-h negative (03, 05)	3	117,29	7,13	142,00 %	2264,41	514,37	238,75 %
03, 05	4	123,58	35,95	149,61 %	2271,82	374,92	239,53 %
06, 07	4	97,90	36,61	81,92 %	2024,08	995,74	173,35 %
09 to 12	8	85,25	25,52	84,89 %	1829,85	915,44	182,51 %
24-h negative total	16	97,99		97,28 %	1988,90		193,04 %
06, 07	4	58,81	13,87	49,21 %	679,95	263,28	58,23 %
09 to 12	8	69,32	17,24	69,03 %	879,61	333,78	87,73 %
24-h positive total	12	65,82		61,64 %	813,06		76,88 %
3-h test 100 (03, 05)	4	97,86	31,01	118,48 %	1664,92	623,97	175,54 %
6-h test 100 (03, 05)	4	102,88	13,74	124,55 %	1615,06	590,12	170,28 %
03, 05	4	114,23	15,14	138,29 %	1883,58	569,57	198,59 %
06, 07	4	112,81	31,19	94,40 %	1766,21	243,85	151,26 %
24-h test 100 total	8	113,52		112,34 %	1824,89		172,48 %
06, 07	4	108,76	8,87	91,01 %	1921,57	285,19	164,57 %
09 to 12	8	96,09	22,25	95,68 %	1640,54	742,13	163,63 %
24-h test 200 total	12	100,31		93,94 %	1734,21		163,98 %
24-h test 400 (06, 07)	4	125,29	4,28	104,84 %	1957,58	702,60	167,65 %
24-h test 1000 (09-12)	8	119,34	32,11	118,84 %	1374,24	796,43	137,07 %
24-h test 5000 (09-12)	8	215,76	86,20	214,86 %	1167,69	692,41	116,47 %

Slice weights (mg)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03	21,0	FALSE	A-02	23,6	21,5
	A-04	21,7	29,4	A-03	45,6	24,8
	A-05	24,2	27,9	A-04	22,5	20,8
	A-06	26,1	26,5	A-05	23,3	25,6
	A-07	19,2	26,1			
mean		22,44	27,475		28,75	23,175
SD		2,72084546	1,49749791		11,2429237	2,3781996
mean-mean	**24,9575**	**repl= 5**		**25,9625**	**repl= 4**	
SE	**3,56028264**	**n= 2**		**3,94212031**	**n= 2**	
neg ctrl 3h	B-01	22,2	23,7	B-01	46,2	24,2
	B-02	22,0	22,7	B-02	21,4	24,4
	B-03	19,0	23,1	B-03	21,5	23,5
	B-04	19,4	23,8	B-04	44,4	26,1
	B-05	26,1	25,7	B-05	42,3	23,0
				B-06	20,9	22,8
mean		21,74	23,8		32,7833333	24,0
SD		2,8404225	1,15325626		12,6777627	1,2083046
mean-mean	**22,77**	**repl= 5**		**28,3916667**	**repl= 6**	
SE	**1,45663997**	**n= 2**		**6,21075456**	**n= 2**	
neg ctrl 6h	B-06	18,1	17,8	B-07	31,0	26,8
	B-07	22,8	23,1	B-08	27,9	21,1
	B-08	23,3	21,2	B-09	FALSE	22,0
	B-09	17,8	23,3	B-10	23,1	25,2
	B-10	23,2	24,9			
mean		21,04	22,06		27,3333333	23,775
SD		2,82895741	2,71900717		3,98036849	2,67628474
mean-mean	**21,55**	**repl= 5**		**25,5541667**	**repl= 4**	
SE	**0,72124892**	**n= 2**		**2,51612163**	**n= 2**	
neg ctrl 24h	B-11	14,3	20,5	B-11	21,2	17,2
	B-12	15,5	17,3	B-12	20,9	18,5
	B-13	18,1	19,2	B-13	16,1	18,0
	B-14	17,0	19,9	B-14	16,4	19,2
	B-15	19,9	19,6	B-15	16,2	19,7
				B-16	16,8	18,3
mean		16,96	19,3		17,9333333	18,4833333
SD		2,1881499	1,21449578		2,42789346	0,88411915
mean-mean	**18,13**	**repl= 5**		**18,2083333**	**repl= 6**	
SE	**1,65462987**	**n= 2**		**0,38890873**	**n= 2**	

Slice weights (mg)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01	22,0	25,9	C-01	22,0	26,4
	C-02	25,8	24,1	C-02	20,7	19,4
	C-03	19,9	29,8	C-03	39,0	26,0
	C-04	21,0	30,1	C-04	20,4	22,7
	C-05	22,7	31	C-05	44,3	23,0
				C-06	20,1	24,9
				C-07	17,5	20,9
mean		22,28	28,18		26,2857143	23,3285714
SD		2,2331592	3,00449663		10,6913405	2,61005382
mean-mean	**25,23**	**repl= 5**		**24,8071429**	**repl= 7**	
SE	**4,17193001**	**n= 2**		**2,09101577**	**n= 2**	
test 6h	C-06	28,8	27,6	C-08	21,5	35,5
	C-07	22,6	24,1	C-09	24,5	23,8
	C-08	22,0	31,7	C-10	28,8	21,7
	C-09	26,0	23,7	C-11	27,4	27,2
	C-10	27,0	27,6	C-12	21,4	25,3
mean		25,28	26,94		24,72	26,7
SD		2,90723236	3,24391738		3,36407491	5,3164838
mean-mean	**26,11**	**repl= 5**		**25,71**	**repl= 5**	
SE	**1,17379726**	**n= 2**		**1,40007143**	**n= 2**	
test 24h	C-11	21,4	23,8	C-13	22,9	17,4
	C-12	21,1	20,8	C-14	23,2	16,0
	C-13	22,6	27,2	C-15	19,1	18,7
	C-14	22,6	23,3	C-16	21,1	19,3
	C-15	23,4	28,3	C-17	18,7	16,7
				C-18	19,0	18,5
				C-19	19,9	21,4
mean		22,22	24,68		20,5571429	18,2857143
SD		0,94973681	3,04909823		1,87958962	1,80132227
mean-mean	**23,45**	**repl= 5**		**19,4214286**	**repl= 7**	
SE	**1,73948268**	**n= 2**		**1,60614255**	**n= 2**	

"menadione" **Negative and positive control (200 µM menadione):**
Mean ± SE from n=2 animals x 5 replicates

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 4-7 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 6-7 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 6-7 replicates

Slice weights (mg)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 0h	A-02	15,1	29,9	A-02	32,2	21,7	28,8	27,5
	A-03	19,3	27,0	A-03	29,9	26,3	31,9	23,1
	A-04	17,1	26,4	A-04	33,4	28,7	26,3	23,9
	A-05	21,7	28,6	A-05	32,9	23,0	25,2	28,0
	A-06	17,6	28,4	A-06	28,5	28,1	25,3	24,8
	A-07	16,6	29,6	A-07	22,2	25,2	26,1	24,4
mean		17,9	28,3166667		29,85	25,5	27,2666667	25,2833333
SD		2,30911238	1,38912442		4,18986873	2,77200289	2,61737782	1,99941658
mean-mean	**23,1083333**	**repl=**	**6**	**26,975**	**repl=**	**6**		
SE	**7,36569564**	**n=**	**2**	**2,11250685**	**n=**	**4**		
neg ctrl 24h	B-02	16,9	23,9	B-02	25,3	16,6	25,0	19,6
	B-03	16,6	24,1	B-03	25,1	17,5	19,5	18,6
	B-04	18,7	21,5	B-04	24,3	19,5	22,2	19,3
	B-05	14,1	23,6	B-05	24,8	15,3	18,3	FALSE
	B-06	15,7	23,1	B-06	21,7	16,6	16,8	19,3
	B-07	14,9	18,8	B-07	23,9	20,6	17,0	18,7
mean		16,15	22,5		24,1833333	17,6833333	19,8	19,1
SD		1,62696036	2,03862699		1,32123679	1,9934058	3,22428287	0,43011626
mean-mean	**19,325**	**repl=**	**6**	**20,1916667**	**repl=**	**6**		
SE	**4,49012806**	**n=**	**2**	**2,80299113**	**n=**	**4**		
pos ctrl 24h	C-02	20,0	26,2	C-02	26,5	20,3	26,2	26,4
	C-03	20,1	33,6	C-03	24,7	22,2	25,6	25,4
	C-04	20,3	32,8	C-04	28,8	24,4	21,6	21,7
	C-05	22,9	29,4	C-05	28,9	20,3	25,1	22,1
	C-06	20,3	29,7	C-06	27,3	20,5	24,5	23,4
	C-07	17,9	24,7	C-07	23,2	24,4	20,3	23,7
mean		20,25	29,4		26,5666667	22,0166667	23,8833333	23,7833333
SD		1,58965405	3,51055551		2,27126984	1,97930964	2,37606117	1,8323937
mean-mean	**24,825**	**repl=**	**6**	**24,0625**	**repl=**	**6**		
SE	**6,47002705**	**n=**	**2**	**1,8767276**	**n=**	**4**		
Fe-100 24h	D-01	19,3	24,5					
	D-02	16,5	21,6					
	D-03	19,9	23,5					
	D-04	13,3	23,9					
	D-05	14,2	24,9					
	D-06	14,8	22,6					
	D-07	18,6	19,4					
mean		16,6571429	22,9142857					
SD		2,64755053	1,91261476					
mean-mean	**19,7857143**	**repl=**	**7**					
SE	**4,42446815**	**n=**	**2**					

Slice weights (mg)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01	17,9	26,5	D-01	23,0	17,5	25,6	20,2
	E-02	19,5	23,1	D-02	25,5	17,2	21,3	17,5
	E-03	16,4	20,7	D-03	20,4	19,3	22,6	19,7
	E-04	19,5	16,2	D-04	24,8	20,8	20,3	18,8
	E-05	16,3	28,6	D-05	24,4	21,2	21,2	18,4
	E-06	16,2	23,5	D-06	17,9	20,1	18,4	19,4
	E-07	15,7	23,7	D-07	29,1	14,3	16,1	19,6
mean		17,3571429	23,1857143		23,5857143	18,6285714	20,7857143	19,0857143
SD		1,61230388	3,99267186		3,62924628	2,44929533	3,02182537	0,91729416
mean-mean	**20,2714286**	**repl=**	**7**	**20,5214286**	**repl=**	**7**		
SE	**4,12142238**	**n=**	**2**	**2,24379719**	**n=**	**4**		
Fe-400 24h	F-01	19,8	26,8					
	F-02	19,3	25,8					
	F-03	16,1	21,9					
	F-04	15,6	23,1					
	F-05	15,2	25,1					
	F-06	17,1	22,2					
	F-07	15,8	22,3					
mean		16,9857143	23,8857143					
SD		1,85241156	1,98110118					
mean-mean	**20,4357143**	**repl=**	**7**					
SE	**4,87903679**	**n=**	**2**					
Fe-1000 24h				E-01	33,6	19,2	23,4	20,3
				E-02	25,0	17,4	FALSE	20,9
				E-03	28,0	21,1	24,0	18,6
				E-04	29,5	18,2	25,0	17,8
				E-05	26,5	16,8	25,5	19,5
				E-06	26,9	20,4	21,7	20,5
				E-07	23,3	15,7	19,2	19,7
mean					27,5428571	18,4	23,1333333	19,6142857
SD					3,33409515	1,95021366	2,34236348	1,09913386
mean-mean				**22,172619**	**repl=**	**7**		
SE				**4,10447532**	**n=**	**4**		
Fe-5000 24h				F-01	24,1	22,6	28,5	20,8
				F-02	30,7	14,5	22,7	20,5
				F-03	25,3	21,8	26,9	18,9
				F-04	25,9	18,7	23,5	19,9
				F-05	25,7	20,0	26,5	21,9
				F-06	26,1	18,3	20,5	18,1
				F-07	29,5	21,0	18,6	20,3
mean					26,7571429	19,5571429	23,8857143	20,0571429
SD					2,39920622	2,72204405	3,60944528	1,25147532
mean-mean				**22,5642857**	**repl=**	**7**		
SE				**3,39876929**	**n=**	**4**		

MTT test (% of 24h negative control; OD-570 / mg slice weight)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl=**	**0**		**repl=**	**0**
SE		**n=**	**0**		**n=**	**0**
neg ctrl 0h	A-03			A-02		
	A-04			A-03		
	A-05			A-04		
	A-06			A-05		
	A-07					
mean						
SD						
mean-mean		**repl=**	**0**		**repl=**	**0**
SE		**n=**	**0**		**n=**	**0**
neg ctrl 3h	B-01			B-01		
	B-02			B-02		
	B-03	0,93713542	0,8729865	B-03		
	B-04	1,23849471	0,80146029	B-04		
	B-05			B-05	0,31268122	0,91059416
				B-06	0,82283465	0,84105195
mean		1,08781507	0,83722339		0,56775794	0,87582306
SD		0,2130932	0,05057666		0,36073295	0,04917377
mean-mean	**0,96251923**	**repl=**	**2**	**0,7217905**	**repl=**	**2**
SE	**0,17719507**	**n=**	**2**	**0,21783493**	**n=**	**2**
neg ctrl 6h	B-06			B-07		
	B-07			B-08		
	B-08			B-09		
	B-09			B-10		
	B-10					
mean						
SD						
mean-mean		**repl=**	**0**		**repl=**	**0**
SE		**n=**	**0**		**n=**	**0**
neg ctrl 24h	B-11			B-11		
	B-12			B-12		
	B-13	1,11895625	1,08577696	B-13		
	B-14	0,88104375	0,91422304	B-14		
	B-15			B-15	0,90203327	0,93175339
				B-16	1,09796673	1,06824661
mean		1,00	1,00		1,00	1,00
SD		0,16822954	0,12130693		0,13854588	0,09651528
mean-mean	**1**	**repl=**	**2**	**1**	**repl=**	**2**
SE	**0**	**n=**	**2**	**0**	**n=**	**2**

MTT test (% of 24h negative control; OD-570 / mg slice weight)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01			C-01		
	C-02			C-02		
	C-03	0,59143821	0,52935792	C-03		
	C-04	0,70115306	0,28857859	C-04		
	C-05			C-05		
				C-06	0,74470865	0,90251697
				C-07	1,07064071	1,04990977
mean		0,64629563	0,40896826		0,90767468	0,97621337
SD		0,07758012	0,17025669		0,23046877	0,10422244
mean-mean	**0,52763195**	**repl=**	**2**	**0,94194402**	**repl=**	**2**
SE	**0,1678158**	**n=**	**2**	**0,04846417**	**n=**	**2**
test 6h	C-06			C-08		
	C-07			C-09		
	C-08			C-10		
	C-09			C-11		
	C-10			C-12		
mean						
SD						
mean-mean		**repl=**	**0**	**#DIV/0!**	**repl=**	**0**
SE		**n=**	**0**	**#DIV/0!**	**n=**	**0**
test 24h	C-11			C-13		
	C-12			C-14		
	C-13	0,14195237	0,35272506	C-15		
	C-14	0,29512286	0,30086187	C-16		
	C-15			C-17		
				C-18	0,48369757	0,71158793
				C-19	0,74854528	0,69915384
mean		0,21853761	0,32679346		0,61612142	0,70537089
SD		0,10830789	0,03667281		0,18727562	0,00879223
mean-mean	**0,27266554**	**repl=**	**2**	**0,66074615**	**repl=**	**2**
SE	**0,07654845**	**n=**	**2**	**0,0631089**	**n=**	**2**

"menadione" **Negative and positive control (200 µM menadione):**
Mean ± SE from n=2 animals x 2 replicates

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 2 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 2 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 2 replicates

MTT test (% of 24h negative control; OD-570 / mg slice weight)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 0h	A-02			A-02				
	A-03			A-03				
	A-04			A-04				
	A-05			A-05				
	A-06	0,92239348	1,13145378	A-06	1,63607229	0,6535595	0,87696926	0,88295862
	A-07	1,07350003	0,94076636	A-07	1,00784749	0,99639621	0,8095143	0,76294354
mean		0,99794675	1,03611007		1,32195989	0,82497786	0,84324178	0,82295108
SD		0,10684847	0,13483637		0,44422202	0,24242216	0,04769786	0,08486348
mean-mean	**1,01702841**	**repl=**	**2**	**0,95328265**	**repl=**	**2**		
SE	**0,02698554**	**n=**	**2**	**0,24595416**	**n=**	**4**		
neg ctrl 24h	B-02			B-02				
	B-03			B-03				
	B-04			B-04				
	B-05			B-05				
	B-06	FALSE	1,10004823	B-06	1,08729614	0,92155026	1,11977981	0,89762424
	B-07	1,00	0,89995177	B-07	0,91270386	1,07844974	0,88022019	1,10237576
mean		1,00	1,00		1,00	1,00	1,00	1,00
SD		#DIV/0!	0,14148957		0,12345538	0,11094468	0,16939424	0,14478119
mean-mean	**1**	**repl=**	**2**	**1**	**repl=**	**2**		
SE	**0**	**n=**	**2**	**0**	**n=**	**4**		
pos ctrl 24h	C-02			C-02				
	C-03			C-03				
	C-04			C-04				
	C-05			C-05				
	C-06	0,22834504	0,49148765	C-06	0,2868791	0,5668712	0,35071548	0,63032164
	C-07	0,30991678	0,41085864	C-07	0,31767825	0,42193645	0,33293856	0,5637911
mean		0,26913091	0,45117315		0,30227868	0,49440383	0,34182702	0,59705637
SD		0,05767993	0,05701331		0,02177829	0,10248434	0,01257018	0,0470442
mean-mean	**0,36015203**	**repl=**	**2**	**0,43389147**	**repl=**	**2**		
SE	**0,1287233**	**n=**	**2**	**0,13672639**	**n=**	**4**		
Fe-100 24h	D-01							
	D-02							
	D-03							
	D-04							
	D-05							
	D-06	1,08816194	1,24867868					
	D-07	0,79851358	0,93991243					
mean		0,94333776	1,09429556					
SD		0,20481232	0,21833071					
mean-mean	**1,01881666**	**repl=**	**2**					
SE	**0,10674328**	**n=**	**2**					

MTT test (% of 24h negative control; OD-570 / mg slice weight)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01			D-01				
	E-02			D-02				
	E-03			D-03				
	E-04			D-04				
	E-05			D-05				
	E-06	0,88445877	1,26511454	D-06	1,37891252	0,75193486	0,87360874	1,0315581
	E-07	1,26943926	0,70155379	D-07	0,26403462	0,94526943	0,68129316	0,96759814
mean		1,07694902	0,98333417		0,82147357	0,84860214	0,77745095	0,99957812
SD		0,27222232	0,39849763		0,78833772	0,13670819	0,13598765	0,04522652
mean-mean	**1,03014159**	**repl=**	**2**	**0,86177619**	**repl=**	**2**		
SE	**0,0661957**	**n=**	**2**	**0,09643301**	**n=**	**4**		
Fe-400 24h	F-01							
	F-02							
	F-03							
	F-04							
	F-05							
	F-06	1,03096557	0,77748077					
	F-07	1,11579185	1,1380556					
mean		1,07337871	0,95776818					
SD		0,05998124	0,25496491					
mean-mean	**1,01557345**	**repl=**	**2**					
SE	**0,08174898**	**n=**	**2**					
Fe-1000 24h				E-01				
				E-02				
				E-03				
				E-04				
				E-05				
				E-06	0,54551371	0,82151021	0,59435039	0,94938501
				E-07	0,98786464	1,2042597	0,68070825	1,05793617
mean					0,76668918	1,01288495	0,63752932	1,00366059
SD					0,31278934	0,27064476	0,06106423	0,07675726
mean-mean				**0,85519101**	**repl=**	**2**		
SE				**0,18449912**	**n=**	**4**		
Fe-5000 24h				F-01				
				F-02				
				F-03				
				F-04				
				F-05				
				F-06	0,4352579	0,89886212	0,62410178	1,00390709
				F-07	0,4225399	0,78099032	0,67535609	0,78375802
mean					0,4288989	0,83992622	0,64972894	0,89383255
SD					0,00899298	0,08334794	0,03624227	0,15566889
mean-mean				**0,70309665**	**repl=**	**2**		
SE				**0,21066168**	**n=**	**4**		

Potassium content (% of 0h negative control; µg K / g slice weight)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03			A-02		
	A-04			A-03	1,00	1,00
	A-05			A-04	1,00	1,00
	A-06			A-05		
	A-07					
mean					1,00	1,00
SD					0	0
mean-mean		**repl= 0**		**1,00**	**repl= 2**	
SE		**n= 0**		**0**	**n= 2**	
neg ctrl 3h	B-01			B-01		
	B-02			B-02	2,84369205	2,52850536
	B-03			B-03	2,02397982	1,75483847
	B-04			B-04		
	B-05			B-05		
				B-06		
mean					2,43383594	2,14167192
SD					0,57962408	0,5470651
mean-mean		**repl= 0**		**2,28775393**	**repl= 2**	
SE		**n= 0**		**0,20659116**	**n= 2**	
neg ctrl 6h	B-06			B-07		
	B-07			B-08	2,76814913	3,33111131
	B-08			B-09	FALSE	1,88348591
	B-09			B-10		
	B-10					
mean					2,76814913	2,60729861
SD					#DIV/0!	1,02362573
mean-mean		**repl= 0**		**2,68772387**	**repl= 2**	
SE		**n= 0**		**0,1137385**	**n= 2**	
neg ctrl 24h	B-11			B-11		
	B-12			B-12	3,21315691	2,48538742
	B-13			B-13	2,52891172	1,84058179
	B-14			B-14		
	B-15			B-15		
				B-16		
mean					2,87103432	2,1629846
SD					0,48383441	0,45594644
mean-mean		**repl= 0**		**2,51700946**	**repl= 2**	
SE		**n= 0**		**0,50066676**	**n= 2**	

Potassium content (% of 0h negative control; µg K / g slice weight)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01			C-01		
	C-02			C-02		
	C-03			C-03	1,3927406	1,87543623
	C-04			C-04	1,9856804	1,65753785
	C-05			C-05		
				C-06		
				C-07		
mean					1,6892105	1,76648704
SD					0,41927175	0,15407743
mean-mean		**repl= 0**		**1,72784877**	**repl= 2**	
SE		**n= 0**		**0,05464276**	**n= 2**	
test 6h	C-06			C-08		
	C-07			C-09		
	C-08			C-10	1,9875898	3,01717731
	C-09			C-11	1,04544184	1,30660899
	C-10			C-12		
mean					1,51651582	2,16189315
SD					0,66619921	1,20955445
mean-mean		**repl= 0**		**1,83920448**	**repl= 2**	
SE		**n= 0**		**0,45635069**	**n= 2**	
test 24h	C-11			C-13		
	C-12			C-14		
	C-13			C-15	3,45093696	2,6514811
	C-14			C-16	0,95037197	1,75522639
	C-15			C-17		
				C-18		
				C-19		
mean					2,20065446	2,20335374
SD					1,76816646	0,63374779
mean-mean		**repl= 0**		**2,2020041**	**repl= 2**	
SE		**n= 0**		**0,00190868**	**n= 2**	

"menadione" **-**

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 2 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 2 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 2 replicates

Potassium content (% of 0h negative control; µg K / g slice weight)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl= 0**			**repl= 0**			
SE		**n= 0**			**n= 0**			
neg ctrl 0h	A-02			A-02				
	A-03	1,00	1,00	A-03	1,00	1,00	1,00	1,00
	A-04	1,00	1,00	A-04	1,00	1,00	1,00	1,00
	A-05			A-05				
	A-06			A-06				
	A-07			A-07				
mean		1,00	1,00		1,00	1,00	1,00	1,00
SD		0	0		0	0	0	0
mean-mean	**1,00**	**repl= 2**		**1,00**	**repl= 2**			
SE	**0**	**n= 2**		**0**	**n= 4**			
neg ctrl 24h	B-02			B-02				
	B-03	1,98992457	0,71924511	B-03	1,07163721	1,99324502	1,56395593	2,04590872
	B-04	2,00130686	1,97331621	B-04	1,24699525	1,71211325	1,71836613	2,46783054
	B-05			B-05				
	B-06			B-06				
	B-07			B-07				
mean		1,99561571	1,34628066		1,15931623	1,85267913	1,64116103	2,25686963
SD		0,0080485	0,88676218		0,12399687	0,19879018	0,1091845	0,29834378
mean-mean	**1,67094819**	**repl= 2**		**1,72750651**	**repl= 2**			
SE	**0,45914922**	**n= 2**		**0,4568695**	**n= 4**			
pos ctrl 24h	C-02			C-02				
	C-03	0,6213064	0,40876518	C-03	0,94423213	0,90412127	0,98846267	1,08206679
	C-04	0,68530236	0,55347848	C-04	0,50020133	0,65897719	1,14801033	0,74195099
	C-05			C-05				
	C-06			C-06				
	C-07			C-07				
mean		0,65330438	0,48112183		0,72221673	0,78154923	1,0682365	0,91200889
SD		0,04525198	0,10232775		0,31397719	0,17334304	0,11281723	0,24049818
mean-mean	**0,56721311**	**repl= 2**		**0,87100284**	**repl= 2**			
SE	**0,12175145**	**n= 2**		**0,15353813**	**n= 4**			
Fe-100 24h	D-01							
	D-02							
	D-03	1,26494088	1,91260665					
	D-04	1,52771516	1,47898122					
	D-05							
	D-06							
	D-07							
mean		1,39632802	1,69579394					
SD		0,18580948	0,30661948					
mean-mean	**1,54606098**	**repl= 2**						
SE	**0,21175438**	**n= 2**						

Potassium content (% of 0h negative control; µg K / g slice weight)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01			D-01				
	E-02			D-02				
	E-03	1,49429169	1,7595139	D-03	1,54105486	1,08679635	1,51084761	1,89646243
	E-04	1,64550126	1,76604662	D-04	1,27157586	1,27484208	1,99329193	2,34150358
	E-05			D-05				
	E-06			D-06				
	E-07			D-07				
mean		1,56989648	1,76278026		1,40631536	1,18081922	1,75206977	2,118983
SD		0,10692131	0,00461933		0,19055043	0,13296841	0,34113965	0,31469162
mean-mean	**1,66633837**	**repl=**	**2**	**1,61454684**	**repl=**	**2**		
SE	**0,13638943**	**n=**	**2**	**0,41022287**	**n=**	**4**		
Fe-400 24h	F-01							
	F-02							
	F-03	1,68617581	1,67795462					
	F-04	2,0106134	1,2078022					
	F-05							
	F-06							
	F-07							
mean		1,84839461	1,44287841					
SD		0,22941202	0,33244796					
mean-mean	**1,64563651**	**repl=**	**2**					
SE	**0,28674325**	**n=**	**2**					
Fe-1000 24h				E-01				
				E-02				
				E-03	0,46065523	1,53457285	1,51905013	1,78500528
				E-04	0,46932132	1,39998184	1,24389947	1,66859963
				E-05				
				E-06				
				E-07				
mean					0,46498828	1,46727734	1,3814748	1,72680245
SD					0,00612785	0,09517022	0,1945609	0,08231123
mean-mean				**1,26013572**	**repl=**	**2**		
SE				**0,55005094**	**n=**	**4**		
Fe-5000 24h				F-01				
				F-02				
				F-03	0,51478297	1,10868491	0,56582619	1,53437017
				F-04	0,77323873	1,18526867	1,356837	1,63906541
				F-05				
				F-06				
				F-07				
mean					0,64401085	1,14697679	0,96133159	1,58671779
SD					0,18275582	0,0541529	0,5593291	0,07403072
mean-mean				**1,08475926**	**repl=**	**2**		
SE				**0,39383877**	**n=**	**4**		

Iron content (% of 0h negative control; µg Fe / g slice weight)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03			A-02		
	A-04			A-03	1,00	1,00
	A-05			A-04	1,00	1,00
	A-06			A-05		
	A-07					
mean					1,00	1,00
SD					0	0
mean-mean		**repl= 0**		**1,00**	**repl= 2**	
SE		**n= 0**		**0**	**n= 2**	
neg ctrl 3h	B-01			B-01		
	B-02			B-02	0,92380452	1,08059278
	B-03			B-03	1,1670096	1,0927364
	B-04			B-04		
	B-05			B-05		
				B-06		
mean					1,04540706	1,08666459
SD					0,17197196	0,00858684
mean-mean		**repl= 0**		**1,06603583**	**repl= 2**	
SE		**n= 0**		**0,02917348**	**n= 2**	
neg ctrl 6h	B-06			B-07		
	B-07			B-08	1,20262835	1,6316027
	B-08			B-09	FALSE	1,60238255
	B-09			B-10		
	B-10					
mean					1,20262835	1,61699263
SD					#DIV/0!	0,02066177
mean-mean		**repl= 0**		**1,40981049**	**repl= 2**	
SE		**n= 0**		**0,29299979**	**n= 2**	
neg ctrl 24h	B-11			B-11		
	B-12			B-12	1,21974876	1,27828933
	B-13			B-13	1,98419527	1,48059354
	B-14			B-14		
	B-15			B-15		
				B-16		
mean					1,60197201	1,37944143
SD					0,54054531	0,14305067
mean-mean		**repl= 0**		**1,49070672**	**repl= 2**	
SE		**n= 0**		**0,15735288**	**n= 2**	

Iron content (% of 0h negative control; µg Fe / g slice weight)

Single-concentration, 3-/6-/24-hour experiments

	"menadione"			"Fe-100"	
	EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01		C-01		
	C-02		C-02		
	C-03		C-03	0,87908133	0,99361655
	C-04		C-04	1,61240133	1,25388149
	C-05		C-05		
			C-06		
			C-07		
mean				1,24574133	1,12374902
SD				0,51853554	0,18403511
mean-mean		**repl= 0**	**1,18474517**	**repl= 2**	
SE		**n= 0**	**0,08626159**	**n= 2**	
test 6h	C-06		C-08		
	C-07		C-09		
	C-08		C-10	1,04250417	1,60791422
	C-09		C-11	1,07894303	1,32668242
	C-10		C-12		
mean				1,0607236	1,46729832
SD				0,02576616	0,19886091
mean-mean		**repl= 0**	**1,26401096**	**repl= 2**	
SE		**n= 0**	**0,28749174**	**n= 2**	
test 24h	C-11		C-13		
	C-12		C-14		
	C-13		C-15	1,34058138	1,63467056
	C-14		C-16	1,04279308	1,59032216
	C-15		C-17		
			C-18		
			C-19		
mean				1,19168723	1,61249636
SD				0,21056813	0,03135905
mean-mean		**repl= 0**	**1,4020918**	**repl= 2**	
SE		**n= 0**	**0,29755699**	**n= 2**	

"menadione" **-**

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 2 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 2 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 2 replicates

Iron content (% of 0h negative control; µg Fe / g slice weight)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl= 0**		**#DIV/0!**	**repl= 0**			
SE		**n= 0**		**#DIV/0!**	**n= 0**			
neg ctrl 0h	A-02			A-02				
	A-03	1,00	1,00	A-03	1,00	1,00	1,00	1,00
	A-04	1,00	1,00	A-04	1,00	1,00	1,00	1,00
	A-05			A-05				
	A-06			A-06				
	A-07			A-07				
mean		1,00	1,00		1,00	1,00	1,00	1,00
SD		0	0		0	0	0	0
mean-mean	**1,00**	**repl= 2**		**1**	**repl= 2**			
SE	**0**	**n= 2**		**0**	**n= 4**			
neg ctrl 24h	B-02			B-02				
	B-03	1,34649731	0,3563585	B-03	0,59657443	1,52145743	0,74830916	0,58128065
	B-04	0,9410322	0,81899807	B-04	0,53247292	1,0777468	1,04074648	0,88327807
	B-05			B-05				
	B-06			B-06				
	B-07			B-07				
mean		1,14376476	0,58767829		0,56452367	1,29960212	0,89452782	0,73227936
SD		0,28670712	0,32713557		0,04532661	0,31375079	0,20678442	0,21354443
mean-mean	**0,86572152**	**repl= 2**		**0,87273324**	**repl= 2**			
SE	**0,39321251**	**n= 2**		**0,31486107**	**n= 4**			
pos ctrl 24h	C-02			C-02				
	C-03	0,68545698	0,30265358	C-03	0,68195516	0,93962621	0,64271071	0,78361417
	C-04	0,56965045	0,47929127	C-04	0,36046184	0,60651652	0,85083455	0,69752202
	C-05			C-05				
	C-06			C-06				
	C-07			C-07				
mean		0,62755371	0,39097242		0,5212085	0,77307136	0,74677263	0,7405681
SD		0,08188758	0,12490171		0,22733011	0,23554412	0,14716578	0,06087634
mean-mean	**0,50926307**	**repl= 2**		**0,69540515**	**repl= 2**			
SE	**0,16728824**	**n= 2**		**0,11698265**	**n= 4**			
Fe-100 24h	D-01							
	D-02							
	D-03	0,91164233	1,03917897					
	D-04	0,9600572	0,82096727					
	D-05							
	D-06							
	D-07							
mean		0,93584977	0,93007312					
SD		0,03423449	0,15429897					
mean-mean	**0,93296144**	**repl= 2**						
SE	**0,00408471**	**n= 2**						

Iron content (% of 0h negative control; µg Fe / g slice weight)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01			D-01				
	E-02			D-02				
	E-03	1,08984821	0,81910846	D-03	0,71936942	1,18563111	0,86409901	0,69105345
	E-04	0,84871518	0,94315242	D-04	0,71119617	0,94279749	1,22985008	1,4720045
	E-05			D-05				
	E-06			D-06				
	E-07			D-07				
mean		0,9692817	0,88113044		0,71528279	1,0642143	1,04697455	1,08152897
SD		0,1705068	0,08771233		0,00577936	0,1717093	0,25862506	0,55221578
mean-mean	**0,92520607**	**repl=**	**2**	**0,97700015**	**repl=**	**2**		
SE	**0,06233235**	**n=**	**2**	**0,17504759**	**n=**	**4**		
Fe-400 24h	F-01							
	F-02							
	F-03	1,22480722	0,83413395					
	F-04	1,000368	1,24130064					
	F-05							
	F-06							
	F-07							
mean		1,11258761	1,03771729					
SD		0,15870249	0,28791033					
mean-mean	**1,07515245**	**repl=**	**2**					
SE	**0,0529413**	**n=**	**2**					
Fe-1000 24h				E-01				
				E-02				
				E-03	0,75664342	1,68851676	0,94710878	1,27002042
				E-04	0,83324079	1,26611219	1,17215115	1,68114058
				E-05				
				E-06				
				E-07				
mean					0,79494211	1,47731447	1,05962997	1,4755805
SD					0,05416252	0,29868514	0,15912898	0,29070585
mean-mean				**1,20186676**	**repl=**	**2**		
SE				**0,33496748**	**n=**	**4**		
Fe-5000 24h				F-01				
				F-02				
				F-03	1,48481385	1,97281934	1,44665347	2,45537411
				F-04	1,50890973	1,45035743	3,27264866	3,82766491
				F-05				
				F-06				
				F-07				
mean					1,49686179	1,71158839	2,35965107	3,14151951
SD					0,01703837	0,36943636	1,29117358	0,97035613
mean-mean				**2,17740519**	**repl=**	**2**		
SE				**0,7400123**	**n=**	**4**		

ALAT leakage (% of total ALAT; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03			A-02		
	A-04			A-03		
	A-05			A-04		
	A-06			A-05		
	A-07					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 3h	B-01	0,03026706	0,03998236	B-01	0,1006239	0,02278351
	B-02	0,06389713	0,03498457	B-02	0,02991521	0,02804124
	B-03	0,0504451	0,03998236	B-03	0,03807391	0,02804124
	B-04	0,04371909	0,03998236	B-04	0,11694129	0,03505155
	B-05	0,0504451	0,04747905	B-05	0,08702608	0,02278351
				B-06	0,03807391	0,04381443
mean		0,0477547	0,04048214		0,06844238	0,03008591
SD		0,01221841	0,00447016		0,03758185	0,00810113
mean-mean	**0,04411842**	**repl= 5**		**0,04926415**	**repl= 6**	
SE	**0,00514248**	**n= 2**		**0,02712212**	**n= 2**	
neg ctrl 6h	B-06	0,0546136	0,06122449	B-07	0,14382403	0,03421122
	B-07	0,06978404	0,06705539	B-08	0,13663283	0,03569866
	B-08	0,06978404	0,06122449	B-09	FALSE	0,03718611
	B-09	0,04854542	0,06997085	B-10	0,08629442	0,05503544
	B-10	0,0546136	0,06997085			
mean		0,05946814	0,06588921		0,12225042	0,04053286
SD		0,00973749	0,0044215		0,03134572	0,00974437
mean-mean	**0,06267868**	**repl= 5**		**0,08139164**	**repl= 4**	
SE	**0,00454038**	**n= 2**		**0,05778305**	**n= 2**	
neg ctrl 24h	B-11	0,0874036	0,16227568	B-11	0,14641148	0,05470041
	B-12	0,08116048	0,11683849	B-12	0,13556619	0,05322202
	B-13	0,0874036	0,12982054	B-13	0,07591707	0,04730846
	B-14	0,09988983	0,13631157	B-14	0,08133971	0,03991651
	B-15	0,11861917	0,17525773	B-15	0,1030303	0,0413949
				B-16	0,07049442	0,03104618
mean		0,09489534	0,1441008		0,10212653	0,04459808
SD		0,01490523	0,02402557		0,03224842	0,00893988
mean-mean	**0,11949807**	**repl= 5**		**0,0733623**	**repl= 6**	
SE	**0,03479352**	**n= 2**		**0,04067876**	**n= 2**	

ALAT leakage (% of total ALAT; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01	0,08545099	0,05464388	C-01	0,03745083	0,02750531
	C-02	0,09969282	0,0460159	C-02	0,04880383	0,0133055
	C-03	0,05696733	0,05751988	C-03	0,21148325	0,0413949
	C-04	0,07120916	0,06902385	C-04	0,02407553	0,02578623
	C-05	0,0617146	0,06614786	C-05	0,09362707	0,02922439
				C-06	0,02942565	0,02234806
				C-07	0,01872541	0,02406715
mean		0,07500698	0,05867028		0,06622737	0,02623308
SD		0,01757134	0,0092301		0,06876199	0,0084415
mean-mean	**0,06683863**	**repl=**	**5**	**0,04623022**	**repl=**	**7**
SE	**0,0115518**	**n=**	**2**	**0,02828023**	**n=**	**2**
test 6h	C-06	0,16714101	0,14250946	C-08	0,10687023	0,06857192
	C-07	0,0923674	0,1563007	C-09	0,09541985	0,03501545
	C-08	0,10116429	0,17468902	C-10	0,1259542	0,0379334
	C-09	0,0923674	0,13791239	C-11	0,09923664	0,05252317
	C-10	0,13635188	0,1563007	C-12	0,08396947	0,05252317
mean		0,1178784	0,15354246		0,10229008	0,04931342
SD		0,03297369	0,01439114		0,01559757	0,01346692
mean-mean	**0,13571043**	**repl=**	**5**	**0,07580175**	**repl=**	**5**
SE	**0,0252183**	**n=**	**2**	**0,03746015**	**n=**	**2**
test 24h	C-11	0,39230769	0,10610789	C-13	0,1827302	0,06371611
	C-12	0,44461538	0,09094962	C-14	0,12472089	0,04435168
	C-13	0,39230769	0,12126616	C-15	0,13556619	0,05174363
	C-14	0,23538462	0,10610789	C-16	0,13400215	0,03660287
	C-15	0,26153846	0,14400357	C-17	0,14009316	0,08133971
				C-18	0,15836618	0,10167464
				C-19	0,13400215	0,05964912
mean		0,34523077	0,11368703		0,14421156	0,0627254
SD		0,09135146	0,02005251		0,01984982	0,02241488
mean-mean	**0,2294589**	**repl=**	**5**	**0,10346848**	**repl=**	**7**
SE	**0,16372615**	**n=**	**2**	**0,05761942**	**n=**	**2**

"menadione" **Negative and positive control (200 µM menadione):**
Mean ± SE from n=2 animals x 5 replicates

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 4-7 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 6-7 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 6-7 replicates

ALAT leakage (% of total ALAT; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 0h	A-02			A-02				
	A-03			A-03				
	A-04			A-04				
	A-05			A-05				
	A-06			A-06				
	A-07			A-07				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 24h	B-02	0,07247535	0,10351508	B-02	0,34860384	0,10016069	0,15632184	0,05352032
	B-03	0,06341593	0,13174647	B-03	0,30410122	0,12747724	0,14850575	0,04865484
	B-04	0,09965361	0,03764185	B-04	0,31893543	0,13658275	0,13287356	0,03892387
	B-05	0,04076739	0,1364517	B-05	0,26701571	0,10016069	0,11724138	0,10704064
	B-06	0,05435651	0,08939939	B-06	0,22251309	0,11837172	0,0937931	0,07298226
	B-07	0,06341593	0,06587324	B-07	0,21509599	0,12747724	0,1016092	0,04865484
mean		0,06568079	0,09410462		0,27937755	0,11837172	0,12505747	0,06162946
SD		0,01979644	0,03822548		0,05382725	0,01523644	0,02520614	0,0249361
mean-mean	**0,07989271**	**repl=**	**6**	**0,14610905**	**repl=**	**6**		
SE	**0,02009869**	**n=**	**2**	**0,09329134**	**n=**	**4**		
pos ctrl 24h	C-02	0,07689145	0,13934426	C-02	0,27419355	0,10559006	0,1162546	0,09451008
	C-03	0,11883224	0,12192623	C-03	0,19707661	0,09503106	0,12519726	0,07088256
	C-04	0,11883224	0,13934426	C-04	0,19707661	0,10559006	0,10731194	0,07088256
	C-05	0,11184211	0,13934426	C-05	0,27419355	0,10559006	0,1162546	0,08269632
	C-06	0,06990132	0,18288934	C-06	0,05997984	0,09503106	0,08942662	0,0886032
	C-07	0,08388158	0,09579918	C-07	0,17993952	0,11614907	0,06259863	0,08269632
mean		0,09669682	0,13644126		0,19707661	0,10383023	0,10284061	0,08171184
SD		0,02228818	0,02844353		0,0787188	0,00794853	0,02314751	0,00946331
mean-mean	**0,11656904**	**repl=**	**6**	**0,12136482**	**repl=**	**6**		
SE	**0,02810356**	**n=**	**2**	**0,05149512**	**n=**	**4**		
Fe-100 24h	D-01	0,0561674	0,16554735					
	D-02	0,0842511	0,14842176					
	D-03	0,12169604	0,12558764					
	D-04	0,0842511	0,15413029					
	D-05	0,07488987	0,19408999					
	D-06	0,10297357	0,12558764					
	D-07	0,09829295	0,12558764					
mean		0,08893172	0,14842176					
SD		0,02110607	0,02574118					
mean-mean	**0,11867674**	**repl=**	**7**					
SE	**0,04206581**	**n=**	**2**					

ALAT leakage (% of total ALAT; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01	0,06726014	0,30167015	D-01	0,28909166	0,1045082	0,1515827	0,05315268
	E-02	0,08407517	0,33716075	D-02	0,27498963	0,16547131	0,15916184	0,07972903
	E-03	0,10089021	0,23956159	D-03	0,21153049	0,1567623	0,14400357	0,05315268
	E-04	0,10509397	0,17745303	D-04	0,31024471	0,13934426	0,09852876	0,04872329
	E-05	0,0504451	0,24843424	D-05	0,3031937	0,13934426	0,14400357	0,06201146
	E-06	0,0714639	0,24843424	D-06	0,21153049	0,11321721	0,12126616	0,06201146
	E-07	0,0504451	0,22181628	D-07	0,28909166	0,09579918	0,10610789	0,07529964
mean		0,07566766	0,25350432		0,26995319	0,13063525	0,1320935	0,06201146
SD		0,0221114	0,05220486		0,04145819	0,02660648	0,02350638	0,01171907
mean-mean	**0,16458599**	**repl=**	**7**	**0,14867335**	**repl=**	**7**		
SE	**0,12574951**	**n=**	**2**	**0,08721496**	**n=**	**4**		
Fe-400 24h	F-01	0,14261745	0,18585583					
	F-02	0,08557047	0,18585583					
	F-03	0,13120805	0,16262385					
	F-04	0,11409396	0,18585583					
	F-05	0,11409396	0,18004783					
	F-06	0,10838926	0,16843184					
	F-07	0,06845638	0,16843184					
mean		0,10920422	0,17672898					
SD		0,02539041	0,00997958					
mean-mean	**0,1429666**	**repl=**	**7**					
SE	**0,04774721**	**n=**	**2**					
Fe-1000 24h				E-01	0,34913112	0,16047198	0,17346939	0,12118126
				E-02	0,28199052	0,15044248	0,1971243	0,10386965
				E-03	0,28199052	0,19056047	0,18923933	0,09809912
				E-04	0,28870458	0,17050147	0,22077922	0,10386965
				E-05	0,24170616	0,15044248	0,22077922	0,10386965
				E-06	0,22827804	0,18053097	0,18135436	0,08655804
				E-07	0,18799368	0,11032448	0,13404453	0,09232858
mean					0,26568495	0,15903919	0,18811291	0,10139657
SD					0,05169639	0,02617204	0,03000042	0,01097775
mean-mean				**0,1785584**	**repl=**	**7**		
SE				**0,06835513**	**n=**	**4**		
Fe-5000 24h				F-01	0,27811861	0,21881188	0,27025641	0,10284061
				F-02	0,3406953	0,11782178	0,34	0,09389795
				F-03	0,25725971	0,21881188	0,30512821	0,08942662
				F-04	0,28507157	0,19356436	0,25282051	0,09389795
				F-05	0,31288344	0,16831683	0,26153846	0,1162546
				F-06	0,25030675	0,14306931	0,18307692	0,06706996
				F-07	0,23640082	0,15990099	0,19179487	0,10284061
mean					0,28010517	0,17432815	0,2578022	0,09517547
SD					0,03672901	0,03817067	0,05646673	0,01521004
mean-mean				**0,20185275**	**repl=**	**7**		
SE				**0,08444215**	**n=**	**4**		

ASAT leakage (% of total ASAT; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03			A-02		
	A-04			A-03		
	A-05			A-04		
	A-06			A-05		
	A-07					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 3h	B-01	0,01063164	0,01838928	B-01	0,02210118	0,02466151
	B-02	0,01771941	0,01660967	B-02	0,00724004	0,02671663
	B-03	0,01366926	0,01660967	B-03	0,00990743	0,03082689
	B-04	0,01366926	0,01779608	B-04	0,02629278	0,03288201
	B-05	0,01366926	0,02016889	B-05	0,02019591	0,01712605
				B-06	0,01028848	0,02397647
mean		0,01387177	0,01791472		0,0160043	0,02603159
SD		0,0025212	0,00147706		0,00783899	0,00558215
mean-mean	**0,01589324**	**repl= 5**		**0,02101795**	**repl= 6**	
SE	**0,0028588**	**n= 2**		**0,00709036**	**n= 2**	
neg ctrl 6h	B-06	0,01468297	0,02442728	B-07	0,06675799	0,02890424
	B-07	0,02029705	0,03690078	B-08	0,07452055	0,02890424
	B-08	0,02418371	0,03274295	B-09	FALSE	0,02951923
	B-09	0,01295556	0,03742051	B-10	0,04657534	0,03628405
	B-10	0,01813779	0,04625591			
mean		0,01805142	0,03554949		0,06261796	0,03090294
SD		0,00447129	0,00792992		0,01442527	0,0035991
mean-mean	**0,02680045**	**repl= 5**		**0,04676045**	**repl= 4**	
SE	**0,012373**	**n= 2**		**0,02242591**	**n= 2**	
neg ctrl 24h	B-11	0,02331816	0,07792293	B-11	0,07649749	0,05338546
	B-12	0,03020762	0,04898013	B-12	0,07148126	0,05393021
	B-13	0,02702787	0,0578856	B-13	0,03699469	0,04739321
	B-14	0,03815699	0,06623449	B-14	0,0351136	0,03867722
	B-15	0,04981607	0,08849818	B-15	0,0526704	0,04412472
				B-16	0,03762172	0,03268497
mean		0,03370534	0,06790427		0,05172986	0,04503263
SD		0,01053671	0,01569847		0,0184212	0,00834493
mean-mean	**0,0508048**	**repl= 5**		**0,04838125**	**repl= 6**	
SE	**0,02418229**	**n= 2**		**0,00473566**	**n= 2**	

ASAT leakage (% of total ASAT; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01	0,02113573	0,01750006	C-01	0,00859767	0,02451573
	C-02	0,02536288	0,01531256	C-02	0,01065949	0,01307399
	C-03	0,0158518	0,01881257	C-03	0,03887577	0,02178998
	C-04	0,01743698	0,01618756	C-04	0,00722204	0,02319055
	C-05	0,01532341	0,01793757	C-05	0,01925878	0,02716608
				C-06	0,00722204	0,02451573
				C-07	0,0051586	0,02385314
mean		0,01902216	0,01715006		0,01385634	0,02258646
SD		0,0042106	0,00139727		0,01194927	0,00450015
mean-mean	**0,01808611**	**repl=**	**5**	**0,0182214**	**repl=**	**7**
SE	**0,00132377**	**n=**	**2**	**0,00617312**	**n=**	**2**
test 6h	C-06	0,05832089	0,04561955	C-08	0,05306512	0,05413582
	C-07	0,03186049	0,05092414	C-09	0,04643198	0,03180479
	C-08	0,04158064	0,06259426	C-10	0,05159109	0,03654168
	C-09	0,03186049	0,04508909	C-11	0,05011706	0,04060186
	C-10	0,04482069	0,06153334	C-12	0,0427469	0,04466205
mean		0,04168864	0,05315208		0,04879043	0,04154924
SD		0,01095032	0,00845747		0,0041822	0,00850057
mean-mean	**0,04742036**	**repl=**	**5**	**0,04516983**	**repl=**	**5**
SE	**0,00810587**	**n=**	**2**	**0,00512029**	**n=**	**2**
test 24h	C-11	0,07572164	0,06237474	C-13	0,11921178	0,05113363
	C-12	0,09833979	0,06132643	C-14	0,08652995	0,04249047
	C-13	0,07670504	0,06866463	C-15	0,06709206	0,05011696
	C-14	0,0481865	0,07233373	C-16	0,09190629	0,02921922
	C-15	0,06982125	0,06761632	C-17	0,07858654	0,05972752
				C-18	0,09989814	0,08164194
				C-19	0,06859672	0,05242272
mean		0,07375484	0,06646317		0,08740307	0,05239321
SD		0,01793185	0,00457551		0,01840702	0,0160787
mean-mean	**0,07010901**	**repl=**	**5**	**0,06989814**	**repl=**	**7**
SE	**0,00515599**	**n=**	**2**	**0,02475571**	**n=**	**2**

"menadione" **Negative and positive control (200 µM menadione):**
Mean ± SE from n=2 animals x 5 replicates

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 4-7 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 6-7 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 6-7 replicates

ASAT leakage (% of total ASAT; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl= 0**			**repl= 0**			
SE		**n= 0**			**n= 0**			
neg ctrl 0h	A-02			A-02				
	A-03			A-03				
	A-04			A-04				
	A-05			A-05				
	A-06			A-06				
	A-07			A-07				
mean								
SD								
mean-mean		**repl= 0**			**repl= 0**			
SE		**n= 0**			**n= 0**			
neg ctrl 24h	B-02	0,04223443	0,07628845	B-02	0,10398703	0,07965451	0,07747721	0,04071682
	B-03	0,03711511	0,07687528	B-03	0,08582062	0,09021113	0,08622464	0,04507934
	B-04	0,06911089	0,05340191	B-04	0,10523989	0,09788868	0,06810497	0,03926265
	B-05	0,0243168	0,08039629	B-05	0,07141278	0,07869482	0,06498089	0,09670245
	B-06	0,0447941	0,05692292	B-06	0,06326922	0,13339731	0,04811085	0,05816689
	B-07	0,0447941	0,03697055	B-07	0,08143563	0,12667946	0,05935754	0,04435225
mean		0,04372757	0,0634759		0,08519419	0,10108765	0,06737602	0,05404673
SD		0,01462409	0,017187		0,01697146	0,02361443	0,01340907	0,02194146
mean-mean	**0,05360174**	**repl= 6**		**0,07692615**	**repl= 6**			
SE	**0,01396418**	**n= 2**		**0,02054922**	**n= 4**			
pos ctrl 24h	C-02	0,0755913	0,06783507	C-02	0,07415644	0,08536526	0,07915473	0,0454237
	C-03	0,08524125	0,07382051	C-03	0,06325108	0,08536526	0,09209348	0,04686572
	C-04	0,0948912	0,08446131	C-04	0,08578882	0,0952151	0,06164936	0,07714819
	C-05	0,08041627	0,07049527	C-05	0,08651584	0,08454444	0,0974212	0,05335482
	C-06	0,0755913	0,07049527	C-06	0,01672155	0,07633625	0,08828797	0,06200696
	C-07	0,08122044	0,06517487	C-07	0,07924561	0,09849838	0,06697708	0,06272797
mean		0,08215863	0,07204705		0,06761322	0,08755412	0,08093064	0,05792123
SD		0,00723598	0,00673859		0,02636201	0,00803677	0,01428497	0,01190578
mean-mean	**0,07710284**	**repl= 6**		**0,0735048**	**repl= 6**			
SE	**0,00714996**	**n= 2**		**0,01329266**	**n= 4**			
Fe-100 24h	D-01	0,04451742	0,06615714					
	D-02	0,06571619	0,07211724					
	D-03	0,10104747	0,07509729					
	D-04	0,05582343	0,07271325					
	D-05	0,0522903	0,08284542					
	D-06	0,06218306	0,06615714					
	D-07	0,0798487	0,0464888					
mean		0,06591808	0,06879661					
SD		0,01909509	0,0113652					
mean-mean	**0,06735734**	**repl= 7**						
SE	**0,00203543**	**n= 2**						

ASAT leakage (% of total ASAT; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01	0,062256	0,09449732	D-01	0,09591062	0,06996886	0,06699727	0,04337912
	E-02	0,06636725	0,10401504	D-02	0,07647775	0,09673956	0,07146376	0,09506488
	E-03	0,07458974	0,06662401	D-03	0,07961208	0,13263663	0,06755558	0,0830664
	E-04	0,0734151	0,0645845	D-04	0,08964195	0,09004688	0,04019836	0,04522504
	E-05	0,03817585	0,10741422	D-05	0,07961208	0,08092051	0,05415613	0,06183832
	E-06	0,06166868	0,10537471	D-06	0,05829861	0,06327619	0,05638937	0,06737608
	E-07	0,03876317	0,08905863	D-07	0,11158229	0,04745714	0,05136458	0,07568272
mean		0,0593194	0,09022406		0,08444791	0,08300654	0,05830358	0,06737608
SD		0,01508473	0,01802407		0,0167766	0,02747328	0,01103921	0,01905713
mean-mean	**0,07477173**	**repl=**	**7**	**0,07328353**	**repl=**	**7**		
SE	**0,0218529**	**n=**	**2**	**0,01262903**	**n=**	**4**		
Fe-400 24h	F-01	0,10406191	0,08652038					
	F-02	0,05351755	0,10658307					
	F-03	0,07432994	0,07460815					
	F-04	0,06466705	0,08840125					
	F-05	0,07655984	0,09717868					
	F-06	0,07507324	0,08714734					
	F-07	0,04757116	0,08589342					
mean		0,07082581	0,08947604					
SD		0,01846674	0,01000987					
mean-mean	**0,08015093**	**repl=**	**7**					
SE	**0,0131877**	**n=**	**2**					
Fe-1000 24h				E-01	0,10370265	0,0935032	0,08833645	0,09584337
				E-02	0,1049085	0,09190485	0,12837383	0,08713034
				E-03	0,09345297	0,13506017	0,08198131	0,07928861
				E-04	0,10068804	0,10149492	0,13028037	0,09148685
				E-05	0,08923252	0,10868748	0,12074766	0,08974425
				E-06	0,08199745	0,11747838	0,08325234	0,09148685
				E-07	0,08018868	0,06313464	0,0591028	0,08974425
mean					0,09345297	0,10160909	0,09886782	0,08924636
SD					0,01011281	0,02258719	0,02755977	0,00512657
mean-mean				**0,09579406**	**repl=**	**7**		
SE				**0,00552617**	**n=**	**4**		
Fe-5000 24h				F-01	0,15882428	0,16499288	0,18632689	0,13847252
				F-02	0,15012158	0,08217792	0,2026305	0,12205567
				F-03	0,13924321	0,15671138	0,24998858	0,12205567
				F-04	0,14141888	0,14715581	0,1451797	0,12276945
				F-05	0,13996843	0,10065203	0,15604877	0,14346895
				F-06	0,13996843	0,15225212	0,11179614	0,11777302
				F-07	0,15664861	0,12294836	0,12577065	0,14132762
mean					0,14659906	0,13241293	0,16824875	0,12970327
SD					0,00848264	0,03131747	0,04806702	0,01086968
mean-mean				**0,144241**	**repl=**	**7**		
SE				**0,0176369**	**n=**	**4**		

GLDH leakage (% of total GLDH; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03			A-02		
	A-04			A-03		
	A-05			A-04		
	A-06			A-05		
	A-07					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 3h	B-01	0,00109373	0,00170968	B-01	0,00156641	0,00252911
	B-02	0,00084133	0,00170968	B-02	0,00113921	0,00234177
	B-03	0,0010096	0,00132111	B-03	0,00142401	0,00206075
	B-04	0,00067306	0,00202053	B-04	0,00185122	0,00290379
	B-05	0,00109373	0,00271994	B-05	0,00170881	0,00177974
				B-06	0,00113921	0,00168607
mean		0,00094229	0,00189619		0,00147148	0,00221687
SD		0,0001824	0,00052305		0,00029414	0,00046522
mean-mean	**0,00141924**	**repl= 5**		**0,00184418**	**repl= 6**	
SE	**0,00067451**	**n= 2**		**0,00052707**	**n= 2**	
neg ctrl 6h	B-06	0,0017866	0,00573471	B-07	0,01010856	0,00455833
	B-07	0,00240802	0,01065018	B-08	0,0140036	0,00546999
	B-08	0,0031848	0,00856483	B-09	FALSE	0,00647282
	B-09	0,00147588	0,01057571	B-10	0,0102013	0,00510532
	B-10	0,00372855	0,01370373			
mean		0,00251677	0,00984583		0,01143782	0,00540162
SD		0,00094116	0,00294132		0,00222252	0,00080645
mean-mean	**0,0061813**	**repl= 5**		**0,00841972**	**repl= 4**	
SE	**0,00518243**	**n= 2**		**0,00426824**	**n= 2**	
neg ctrl 24h	B-11	0,00958074	0,0297144	B-11	0,03609736	0,01912649
	B-12	0,01471328	0,02253569	B-12	0,03844616	0,02332499
	B-13	0,01631007	0,02826049	B-13	0,01792506	0,02117909
	B-14	0,02326752	0,03643876	B-14	0,01743057	0,01343519
	B-15	0,03387477	0,04298138	B-15	0,02324076	0,01520789
				B-16	0,01854316	0,0132486
mean		0,01954928	0,03198615		0,02528051	0,01758704
SD		0,00938477	0,00789103		0,00954584	0,00424069
mean-mean	**0,02576771**	**repl= 5**		**0,02143378**	**repl= 6**	
SE	**0,00879419**	**n= 2**		**0,0054401**	**n= 2**	

GLDH leakage (% of total GLDH; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01	0,00173538	0,00185333	C-01	0,0015484	0,00275652
	C-02	0,00163897	0,00163093	C-02	0,00210156	0,0014928
	C-03	0,00134974	0,0024464	C-03	0,00519209	0,001866
	C-04	0,00125333	0,00192746	C-04	0,00098534	0,00176417
	C-05	0,0009641	0,0015568	C-05	0,00140763	0,00220521
				C-06	0,00098534	0,00231548
				C-07	0,0007742	0,00231548
mean		0,00138831	0,00188298		0,00185637	0,00210224
SD		0,00030941	0,00035008		0,00153687	0,00042252
mean-mean	**0,00163564**	**repl= 5**		**0,0019793**	**repl= 7**	
SE	**0,00034979**	**n= 2**		**0,00017386**	**n= 2**	
test 6h	C-06	0,00337946	0,00675208	C-08	0,01199527	0,01256104
	C-07	0,00231226	0,0089162	C-09	0,01141485	0,00679996
	C-08	0,00435772	0,01038781	C-10	0,00938339	0,00821662
	C-09	0,00222333	0,00605956	C-11	0,01141485	0,00604441
	C-10	0,00275693	0,0126385	C-12	0,00851277	0,01067216
mean		0,00300594	0,00895083		0,01054423	0,00885884
SD		0,00088353	0,00268603		0,00150797	0,00271877
mean-mean	**0,00597838**	**repl= 5**		**0,00970153**	**repl= 5**	
SE	**0,00420367**	**n= 2**		**0,00119175**	**n= 2**	
test 24h	C-11	0,0295475	0,01715439	C-13	0,04286748	0,02157478
	C-12	0,04992508	0,01882799	C-14	0,03807529	0,01828679
	C-13	0,04924583	0,01840959	C-15	0,02979268	0,02043269
	C-14	0,03498152	0,02635918	C-16	0,0456877	0,01267623
	C-15	0,04584957	0,01799119	C-17	0,03169937	0,02224636
				C-18	0,04523647	0,03156465
				C-19	0,03090971	0,01830078
mean		0,0419099	0,01974847		0,03775267	0,02072604
SD		0,00914346	0,00374696		0,00697695	0,0057333
mean-mean	**0,03082918**	**repl= 5**		**0,02923936**	**repl= 7**	
SE	**0,0156705**	**n= 2**		**0,01203965**	**n= 2**	

"menadione" **Negative and positive control (200 µM menadione):**
Mean ± SE from n=2 animals x 5 replicates

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 4-7 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 6-7 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 6-7 replicates

GLDH leakage (% of total GLDH; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 0h	A-02			A-02				
	A-03			A-03				
	A-04			A-04				
	A-05			A-05				
	A-06			A-06				
	A-07			A-07				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 24h	B-02	0,02376665	0,04075961	B-02	0,05634085	0,22340812	0,05055339	0,01682605
	B-03	0,02396806	0,02854254	B-03	0,04107849	0,26413998	0,06532779	0,0175223
	B-04	0,0525686	0,02119067	B-04	0,06332257	0,19872214	0,04111913	0,01415709
	B-05	0,01047344	0,03535382	B-05	0,04643655	0,23328251	0,04254317	0,04490816
	B-06	0,03081608	0,02324487	B-06	0,02955053	0,50235969	0,03097285	0,02645752
	B-07	0,03464291	0,01351446	B-07	0,03555806	0,29376316	0,03684701	0,01821855
mean		0,02937262	0,02710099		0,04538118	FALSE	0,04456055	0,02301495
SD		0,01404086	0,00991101		0,012718	0,11105308	0,0120601	0,01149872
mean-mean	**0,02823681**	**repl=**	**6**	**0,03765223**	**repl=**	**6**		
SE	**0,00160628**	**n=**	**2**	**0,01268289**	**n=**	**3**		
pos ctrl 24h	C-02	0,02636732	0,02055146	C-02	0,01116931	0,02896979	0,02400195	0,00957682
	C-03	0,02131826	0,02926527	C-03	0,01613345	0,03064573	0,03000244	0,00976835
	C-04	0,02393629	0,03797909	C-04	0,02047707	0,03806774	0,01615516	0,03294425
	C-05	0,02318828	0,02745675	C-05	0,02761302	0,02992747	0,03123331	0,01302447
	C-06	0,01570819	0,02235998	C-06	0,01023853	0,0229843	0,02661755	0,02624048
	C-07	0,01870022	0,02318204	C-07	0,01861552	0,02920921	0,01877076	0,01819595
mean		0,02153643	0,0267991		0,01737448	0,02996737	0,02446353	0,01829172
SD		0,0038441	0,00637697		0,00643366	0,00482516	0,00604647	0,00953132
mean-mean	**0,02416776**	**repl=**	**6**	**0,02252428**	**repl=**	**6**		
SE	**0,00372127**	**n=**	**2**	**0,00587637**	**n=**	**4**		
Fe-100 24h	D-01	0,03124426	0,03293506					
	D-02	0,04764749	0,04011157					
	D-03	0,09607609	0,04305906					
	D-04	0,05597929	0,02832159					
	D-05	0,04061753	0,04293091					
	D-06	0,04139864	0,03524179					
	D-07	0,0650922	0,02191399					
mean		0,05400793	0,03493057					
SD		0,02157705	0,0078963					
mean-mean	**0,04446925**	**repl=**	**7**					
SE	**0,01348973**	**n=**	**2**					

GLDH leakage (% of total GLDH; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01	0,03293415	0,0501071	D-01	0,06633427	0,03492285	0,03767819	0,0340982
	E-02	0,03964296	0,05461985	D-02	0,04984768	0,05309537	0,04110348	0,09581595
	E-03	0,03695944	0,03501272	D-03	0,04325305	0,0993958	0,03753547	0,08047176
	E-04	0,03293415	0,02941069	D-04	0,06167924	0,04835471	0,02012358	0,04500963
	E-05	0,01671103	0,06193362	D-05	0,05256312	0,04740658	0,02882952	0,07092426
	E-06	0,03256822	0,06193362	D-06	0,03219734	0,03587098	0,03753547	0,04876043
	E-07	0,01646708	0,04777292	D-07	0,07292891	0,02291318	0,02997129	0,07637997
mean		0,02974529	0,04868436		0,0541148	0,04885135	0,03325386	0,06449432
SD		0,00934984	0,01256688		0,01402067	0,02453015	0,00730992	0,02224885
mean-mean	**0,03921482**	**repl=**	**7**	**0,05017858**	**repl=**	**7**		
SE	**0,01339194**	**n=**	**2**	**0,01302103**	**n=**	**4**		
Fe-400 24h	F-01	0,1426938	0,04431615					
	F-02	0,06670825	0,06008508					
	F-03	0,09100595	0,03452853					
	F-04	0,07775266	0,04513178					
	F-05	0,12060497	0,05002559					
	F-06	0,10779346	0,04268488					
	F-07	0,06626647	0,03942234					
mean		0,09611794	0,04517062					
SD		0,02893873	0,00815699					
mean-mean	**0,07064428**	**repl=**	**7**					
SE	**0,03602519**	**n=**	**2**					
Fe-1000 24h				E-01	0,07988264	0,05317751	0,05198403	0,24712759
				E-02	0,07623318	0,05487919	0,08806706	0,18853032
				E-03	0,07420571	0,08274421	0,0420459	0,17918873
				E-04	0,08353211	0,06126049	0,07614131	0,21570586
				E-05	0,06974525	0,07508665	0,07400079	0,23269058
				E-06	0,05190344	0,06913077	0,04556247	0,22504746
				E-07	0,04257704	0,03722426	0,03042595	0,23523829
mean					0,06829705	0,06192901	0,05831822	0,21764697
SD					0,01525666	0,01524971	0,0211904	0,02513371
mean-mean				**0,10154781**	**repl=**	**7**		
SE				**0,0775093**	**n=**	**4**		
Fe-5000 24h				F-01	0,17387843	0,15629057	0,13121486	0,18969932
				F-02	0,15175318	0,07179111	0,13121486	0,16029305
				F-03	0,14420456	0,12864431	0,16228245	0,18047382
				F-04	0,13821773	0,14068381	0,08662374	0,17960893
				F-05	0,13275149	0,08405356	0,10215753	0,18191531
				F-06	0,12181902	0,14937901	0,06432818	0,17038343
				F-07	0,14524575	0,1103621	0,07967922	0,17730256
mean					0,14398145	0,12017207	0,1082144	0,17709663
SD					0,01636247	0,03263843	0,03469989	0,00937403
mean-mean				**0,13736614**	**repl=**	**7**		
SE				**0,03037397**	**n=**	**4**		

LDH leakage (% of total LDH; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
untreated	A-01			A-01		
	A-02					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 0h	A-03			A-02		
	A-04			A-03		
	A-05			A-04		
	A-06			A-05		
	A-07					
mean						
SD						
mean-mean		**repl= 0**			**repl= 0**	
SE		**n= 0**			**n= 0**	
neg ctrl 3h	B-01	0,06748772	0,07060782	B-01	0,13621795	0,07405707
	B-02	0,09711104	0,06096535	B-02	0,05128958	0,08377209
	B-03	0,07550606	0,06843049	B-03	0,05653365	0,09014259
	B-04	0,06704225	0,06267611	B-04	0,16576381	0,10288359
	B-05	0,07238782	0,06905259	B-05	0,12662514	0,0641828
				B-06	0,06164981	0,07644601
mean		0,07590698	0,06634647		0,09967999	0,08191402
SD		0,01236552	0,0042501		0,04914808	0,01354187
mean-mean	**0,07112672**	**repl= 5**		**0,09079701**	**repl= 6**	
SE	**0,0067603**	**n= 2**		**0,01256243**	**n= 2**	
neg ctrl 6h	B-06	0,08193406	0,09379873	B-07	0,20435061	0,12331547
	B-07	0,12369108	0,10297469	B-08	0,20625235	0,10883019
	B-08	0,13181677	0,09379873	B-09	FALSE	0,10902079
	B-09	0,08509405	0,11384991	B-10	0,14574244	0,16048165
	B-10	0,09683116	0,12472512			
mean		0,10387342	0,10582944		0,18544847	0,12541202
SD		0,02267799	0,01340709		0,03439957	0,02434409
mean-mean	**0,10485143**	**repl= 5**		**0,15543025**	**repl= 4**	
SE	**0,00138311**	**n= 2**		**0,04245218**	**n= 2**	
neg ctrl 24h	B-11	0,10902486	0,29413165	B-11	0,28918237	0,16673057
	B-12	0,13832922	0,17917745	B-12	0,25793905	0,17261765
	B-13	0,12414969	0,21047953	B-13	0,13369235	0,14213098
	B-14	0,18181313	0,24474992	B-14	0,13151259	0,11248531
	B-15	0,22151582	0,32570358	B-15	0,18092063	0,1406592
				B-16	0,130786	0,10260343
mean		0,15496654	0,25084843		0,18733883	0,13953786
SD		0,04606154	0,05975011		0,07012906	0,02806311
mean-mean	**0,20290748**	**repl= 5**		**0,16343834**	**repl= 6**	
SE	**0,06779873**	**n= 2**		**0,03380039**	**n= 2**	

LDH leakage (% of total LDH; U / litre)

Single-concentration, 3-/6-/24-hour experiments

		"menadione"			"Fe-100"	
		EGN-04-00	EGN-02-01		EGN-03-01	EGN-05-01
test 3h	C-01	0,14334666	0,08358602	C-01	0,05366472	0,09375101
	C-02	0,178755	0,07945655	C-02	0,0784716	0,06643992
	C-03	0,11279269	0,09583201	C-03	0,28288527	0,11458784
	C-04	0,11050828	0,08743069	C-04	0,05101927	0,09356826
	C-05	0,10908053	0,09939189	C-05	0,13567348	0,10215753
				C-06	0,05593224	0,09576126
				C-07	0,03250117	0,08479623
mean		0,13089663	0,08913943		0,09859254	0,09300887
SD		0,03026882	0,00832799		0,08776232	0,01489489
mean-mean	**0,11001803**	**repl=**	**5**	**0,0958007**	**repl=**	**7**
SE	**0,0295268**	**n=**	**2**	**0,00394825**	**n=**	**2**
test 6h	C-06	0,4001109	0,26013081	C-08	0,18001263	0,18080039
	C-07	0,21720306	0,29811963	C-09	0,15325646	0,10478421
	C-08	0,25751498	0,35255484	C-10	0,20276448	0,10630835
	C-09	0,21269053	0,27101785	C-11	0,17819249	0,14993668
	C-10	0,28308598	0,28676931	C-12	0,14688594	0,13393327
mean		0,27412109	0,29371849		0,1722224	0,13515258
SD		0,07622818	0,03596119		0,02253215	0,03185124
mean-mean	**0,28391979**	**repl=**	**5**	**0,15368749**	**repl=**	**5**
SE	**0,01385746**	**n=**	**2**	**0,02621232**	**n=**	**2**
test 24h	C-11	0,60076839	0,49600685	C-13	0,49269763	0,20072516
	C-12	0,73927732	0,42042213	C-14	0,37661524	0,12720302
	C-13	0,65819892	0,51083856	C-15	0,25043097	0,16504854
	C-14	0,69198159	0,48716486	C-16	0,36572433	0,11687287
	C-15	0,65819892	0,52082145	C-17	0,29824561	0,23434612
				C-18	0,34228706	0,33801076
				C-19	0,27042996	0,21193215
mean		0,66968503	0,48705077		0,34234726	0,19916266
SD		0,05087629	0,03945224		0,08153903	0,07505081
mean-mean	**0,5783679**	**repl=**	**5**	**0,27075496**	**repl=**	**7**
SE	**0,12914192**	**n=**	**2**	**0,1012468**	**n=**	**2**

"menadione" **Negative and positive control (200 µM menadione):**
Mean ± SE from n=2 animals x 5 replicates

"Fe-100" **Negative control and 100 µM FeSO4:**
Mean ± SE from n=2 animals x 4-7 replicates

"Fe-3xLow" **100-200-400 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=2 animals x 6-7 replicates

"Fe-3xHigh" **200-1000-5000 µM FeSO4 and pos.ctrl. (200 µM menadione):**
Mean ± SE from n=4 animals x 6-7 replicates

LDH leakage (% of total LDH; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
untreated	A-01			A-01				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 0h	A-02			A-02				
	A-03			A-03				
	A-04			A-04				
	A-05			A-05				
	A-06			A-06				
	A-07			A-07				
mean								
SD								
mean-mean		**repl=**	**0**		**repl=**	**0**		
SE		**n=**	**0**		**n=**	**0**		
neg ctrl 24h	B-02	0,20370457	0,27652465	B-02	0,29852428	0,23956736	0,3156464	0,09431633
	B-03	0,16201065	0,31048382	B-03	0,24915954	0,27098603	0,30024265	0,10400163
	B-04	0,26525273	0,20456356	B-04	0,26504347	0,38380762	0,30207022	0,08370863
	B-05	0,12547884	0,22093673	B-05	0,24152903	0,23385488	0,24071628	0,2121541
	B-06	0,18385032	0,20759563	B-06	0,1934101	0,33346634	0,17152993	0,11806837
	B-07	0,20529291	0,11117585	B-07	0,24090613	0,40879975	0,2190466	0,08486164
mean		0,19093167	0,22188004		0,24809543	0,311747	0,25820868	0,11618511
SD		0,04703968	0,06869771		0,03439979	0,07486286	0,05717104	0,04873634
mean-mean	**0,20640586**	**repl=**	**6**	**0,23355905**	**repl=**	**6**		
SE	**0,0218838**	**n=**	**2**	**0,08308411**	**n=**	**4**		
pos ctrl 24h	C-02	0,44400741	0,46941058	C-02	0,57637888	0,46836805	0,48540107	0,24310706
	C-03	0,47726374	0,57732413	C-03	0,49196433	0,49080278	0,45464479	0,2826195
	C-04	0,45745146	0,55456321	C-04	0,57577376	0,560523	0,39617741	0,34867464
	C-05	0,51052007	0,59928173	C-05	0,63265524	0,44938482	0,49270949	0,29739896
	C-06	0,46134316	0,46619727	C-06	0,49620019	0,4735453	0,42297496	0,19394273
	C-07	0,41499657	0,45628958	C-07	0,52222044	0,54257522	0,37151148	0,28442923
mean		0,4609304	0,52051108		0,54919881	0,4975332	0,43723653	0,27502868
SD		0,03204525	0,06368318		0,05517686	0,04424046	0,04880399	0,05227632
mean-mean	**0,49072074**	**repl=**	**6**	**0,4397493**	**repl=**	**6**		
SE	**0,04212991**	**n=**	**2**	**0,1189641**	**n=**	**4**		
Fe-100 24h	D-01	0,15233657	0,31246907					
	D-02	0,25021282	0,29708598					
	D-03	0,34161477	0,29324021					
	D-04	0,22660065	0,28170289					
	D-05	0,18813567	0,38241407					
	D-06	0,22050719	0,27088665					
	D-07	0,30581567	0,19805732					
mean		0,24074619	0,2908366					
SD		0,06543695	0,05475498					
mean-mean	**0,2657914**	**repl=**	**7**					
SE	**0,03541926**	**n=**	**2**					

LDH leakage (% of total LDH; U / litre)

Three-concentration, 24-hour experiments

		"Fe-3xLow"			"Fe-3xHigh"		"Fe-3xHigh"	
		EGN-06-01	EGN-07-01		EGN-09-01	EGN-10-01	EGN-11-01	EGN-12-01
Fe-200 24h	E-01	0,13408381	0,42579748	D-01	0,39603408	0,22347601	0,36167959	0,15593892
	E-02	0,15976469	0,38563865	D-02	0,4007785	0,30600146	0,35638414	0,2782092
	E-03	0,1716174	0,30389693	D-03	0,32187133	0,327424	0,340233	0,23966748
	E-04	0,20100225	0,25918107	D-04	0,3847973	0,327424	0,20466935	0,13334549
	E-05	0,09013	0,55367913	D-05	0,37955347	0,27484141	0,27483413	0,1869495
	E-06	0,15531992	0,3964616	D-06	0,25320212	0,23540446	0,22770458	0,25561578
	E-07	0,0767957	0,42693674	D-07	0,51689189	0,16602466	0,20625798	0,24764163
mean		0,14124483	0,39308452		0,37901838	0,26579943	0,28168039	0,21390972
SD		0,04444516	0,09483156		0,08052054	0,06050243	0,07069704	0,05515612
mean-mean	**0,26716467**	**repl=**	**7**	**0,28510198**	**repl=**	**7**		
SE	**0,17807755**	**n=**	**2**	**0,06897566**	**n=**	**4**		
Fe-400 24h	F-01	0,31137443	0,45746877					
	F-02	0,1349869	0,39800854					
	F-03	0,21013425	0,30667549					
	F-04	0,17847495	0,40015125					
	F-05	0,23379175	0,4025618					
	F-06	0,18543304	0,39158041					
	F-07	0,12489768	0,30988956					
mean		0,19701328	0,38090512					
SD		0,06341529	0,05425427					
mean-mean	**0,2889592**	**repl=**	**7**					
SE	**0,13003116**	**n=**	**2**					
Fe-1000 24h				E-01	0,7254983	0,27554075	0,38925246	0,32879121
				E-02	0,52612687	0,29666234	0,46141725	0,22417582
				E-03	0,52753288	0,36674761	0,30615362	0,20293811
				E-04	0,60317592	0,34498598	0,44528951	0,21748988
				E-05	0,48788355	0,31778393	0,43271534	0,26153846
				E-06	0,44654702	0,3465861	0,34715634	0,25052632
				E-07	0,42517575	0,19905499	0,26515091	0,21277039
mean					0,5345629	0,30676595	0,37816221	0,24260431
SD					0,10261251	0,05692738	0,07467113	0,04336522
mean-mean				**0,36552384**	**repl=**	**7**		
SE				**0,12555959**	**n=**	**4**		
Fe-5000 24h				F-01	0,50982099	0,39414939	0,45235033	0,18408858
				F-02	0,57763648	0,21499058	0,24398177	0,13820464
				F-03	0,49673983	0,38356274	0,42454702	0,12106727
				F-04	0,49329742	0,34040175	0,32582969	0,12714827
				F-05	0,54803175	0,28719701	0,43548091	0,17662553
				F-06	0,53804876	0,31868553	0,24648094	0,14539128
				F-07	0,58624251	0,29099735	0,25803962	0,1727558
mean					0,53568825	0,31856919	0,34095861	0,15218305
SD					0,03754197	0,06176049	0,09466131	0,02541355
mean-mean				**0,33684978**	**repl=**	**7**		
SE				**0,15704522**	**n=**	**4**		